

President Bill Clinton is still in office

The Republican majorities in both houses of the US Congress will complicate the president's life, but do not necessarily presage disaster and may even bring out the best in the present incumbent.

REPORTS that President Bill Clinton is dead in the water now that his political opponents have captured both the Senate and the House of Representatives are exaggerated. That view underestimates the great power of the president of the United States, for which the famous checks and balances of the US Constitution are intended as antidotes. Last Tuesday's mid-term elections were a vivid illustration of one of them in action, but the presidency also retains its constitutional clout.

The president can veto bills that the Congress foists on him if he does not like them. As the *de facto* Chief Executive Officer of USA Inc., he can also use the vast bureaucracy in Washington and beyond to direct the funds that the Congress has appropriated in ways that make sense to him and his advisers. There is also a vast range of government policy, including most of foreign affairs, in which the president and the Secretary of State make the running (although Senator Jesse Helms, almost certain to be the chairman of the Senate Foreign Relations Committee from next January, will want to have a say). Where Clinton will be hurt is in persuading the Congress to appropriate funds for causes it disapproves of. If the new Congress should take the knife to welfare spending, or to the budget of the National Institutes of Health (NIH), Clinton has no constitutional means of preventing it from doing so, but only persuasion and patronage.

The causes of last week's upheaval are not easily identified, but may be almost as many as the number of those who voted (fewer than 40 per cent of those eligible). But the theory that the United States has turned against incumbent politicians is plainly false; no Republican 'rascals' were turned out. If there is substance in the belief that the vote was directed against Washington as such, it is ironical that Clinton should have been punished; his election two years ago owed much to his freedom from association with that place. And in reality, he has much to boast about. He won ratification of the North American Free Trade Agreement, pushed through the latest round of the General Agreement on Tariffs and Trade (GATT), acted energetically and diplomatically in resolving (for the time being) the problem of North Korea's nuclear weapons. He broke his head on health-care reform in the last Congress, but there will still be more than 30 million people without medical insurance after last Tuesday's vote.

What will (or should) happen now? There is some immediate housekeeping to be done; the GATT treaty will need Clinton's energetic intervention if it is to make its way safely

through the fag-end of this Congress. The reform of health-care arrangements in the United States is dead for the time being, but the administration (with the consent of the Congress) could yet salvage some of the ingredients of the abortive battles of the past two years. And presumably even the Republicans soon to be in charge would not cavil at a drive to make health-care in the United States more easily affordable. Is that an extra task for NIH?

But the two years ahead will mostly be a long argument about budgets (see page 207), both in detail and in the large. Some of the wilder plans the Republicans have been advertising, the abolition of overhead payments accompanying federal research grants, for example, are too fanciful to be taken seriously; the Grand Old Party is unlikely to seek a reputation for killing off the institution of the research university, for example. And further attrition of the research supported by the federal agencies can only attenuate the strength of the whole research enterprise in the United States at a time when even Republicans are crying for a more competitive economy.

The chronic federal deficit is the other budget problem that will occupy the next two years. This is a creation of the Reagan years; Clinton, as it happens, has presided over a decline, but one too slow to prevent the decline in value of the dollar against the yen in particular. What has happened in the past few years is that financial institutions outside the United States have shrunk from their previous practice of investing in dollar securities and thus helping to fund the deficit. The newly elected Republicans have been promising to make the problem go away by cutting spending, but even they may not have a knife that is sharp enough, or the daring to shoulder, just two years from now, the blame that will be attached to them by last week's electors. Clinton could easily call their bluff, but that will not solve the problem either. Only a modicum of national impoverishment by means of taxes can do that.

But will not Clinton follow the precedent set by his immediate predecessor of throwing his energy into foreign affairs as a relief from frustration in Washington? Luckily, that is unlikely, and for two reasons. First, he is self-consciously not good at international diplomacy. Last week's pull-out from the arms embargo of Bosnia, coupled with the justification that there were very few US naval vessels in the area, was almost calculated to confirm governments such as the French in their belief that the United States is too self-interested an ally. (That decision, by itself, could well trigger



the end of NATO as a cooperative defence organization even though the treaty will not be torn up.) Second, Clinton seems of a temperament to relish appeals to the American people over the heads of Congress. There will probably be a lot of that in the months to come. □

Science in schools

British arguments about the amount of time for science in schools should instead be about the quality of teaching.

THE research establishment in Britain has reacted ambivalently to the publication last week of the latest (and, it must be hoped) the last version of the national curriculum for the education of young people of 16 and younger. Some regret that it will be possible for young people to emerge from secondary schools (which they may quit, if they choose, at 16) while having studied a form of combined science course for only ten per cent of the time they have spent in classrooms, and have been urging that school students should be persuaded to elect for the double dose of science which is an option under the scheme. Others, by contrast, are plainly relieved that science will remain a part of everybody's education (see page 211). Both opinions miss the realities of secondary school education as it has become.

The reality of modern life in most modern countries is that secondary education is a general education, a preparation for adult life, but not for a particular career. That is how it should be. The core of the government's national curriculum, compulsory for all students, consists of English, mathematics and science, which again is right and proper. But there also has to be time in the school day for young people to learn all the other things they will need to know about as adults, history, foreign languages and music among other things.

Arguments about the proportion of the time that should be spent on science studies is irrelevant. What matters is that all young people should understand something of the nature of scientific inquiry, that they should appreciate its potentially vast scope and that they should acquire a respect for the process. The national curriculum, which is merely a printed document, will not ensure that for all school students, nor will the evaluations and the examinations that accompany it. Everything will depend on the school the students attend and the teachers they find there. It must be hoped that, with the dust now settling, the quality of teaching will improve.

What does that mean for the recruitment of people to the research professions? Nothing, which is how it should be. It is ridiculous to pretend, as some do, that what students learn in the early years of secondary schooling is relevant to their preparation for a career in research, except that bad teaching has the negative effect of turning young people's interests in other directions. Of course, good schools will increasingly be aware that what their students do outside the bounds of the national curriculum may have an important bearing on their futures, in music or in mathematics, but that is again to say that schools matter more than the formal curriculum. □

Russia's backward step

The Russian government is about to institutionalize applied research disastrously.

THE Russian government seems about to make a classic mistake in the administration of science. The report on page 208 that a decree has been signed in conditions of some secrecy for the creation of an academy of applied science is ominous, notably for the people of Russia and for the eventual well-being of Russian industry. The plan seems to be that there will be an academy modelled on, and under the tutelage of, the Russian Academy of Sciences, which will finance and otherwise regulate the myriad research establishments operated by ex-Soviet ministries (including the military establishments). The recipe now being contemplated has all the makings of disaster.

Nobody, of course, denies that there is a problem. The former Soviet Union used to claim that it employed about 4 million people in research establishments, roughly the establishment of the Red Army at its peak. The workforce of the Academy of Sciences was a comparatively small part of the total, probably less than 5 per cent. Since 1987, it has been plain that most of these research workers and their attendants were surplus to any reasonable requirement. Worse than that, even when the applied research establishments were reasonably well equipped, they were constitutionally wrongly sited. The whole world except Russia seems to know that applied research, intended to be helpful to industry, is best planned by individual industrial enterprises and carried out close to and preferably inside the perimeter fences of the relevant industrial plants.

It is an act of madness that the Russian government should now be thinking of perpetuating the old organization, but with the added handicap that the staid forms and organizations of the Russian Academy of Sciences should be grafted onto them. The immediate need appears to offer some security to the people concerned, as well as to invent research programmes on which they can be engaged. No doubt there will be no shortage of schemes on which work could begin quite soon, when the funds for equipment and the like have been raised. But who can have confidence that the programmes will yield results of value to Russian industry, let alone that they will be worthwhile in a more abstract sense?

The long-term price that Russia will have to pay for this new arrangement is even greater. The indifference of working industry to the benefits of research, everywhere apparent, have nowhere been as conspicuous as in Russia. Stalin's Soviet Union specialized in the manufacture of goods and machines that were glaringly inappropriate to the needs of those who would use them. And despite the care spent on the design and monitoring of applied research programmes, Russian civilian industry has mostly been conspicuous for its backwardness in innovation. To institutionalize a system that will perpetuate that state of affairs is at best a folly. When President Boris Yeltsin gets to hear of this decree, he should tear it up. □

US technology research most likely victim of Congressional elections . . .

Washington. US research into nuclear weapons and other military systems is likely to receive a boost as a result of last week's election of Republican majorities in both the House of Representatives and the Senate. But science policy experts expect that the consequences will be a general squeeze on the rest of the science budget — and perhaps the end of the Clinton administration's fledgling technology policy.

Biomedical research could be an exception, at least in budget terms. It has broad political support, and may therefore be immune from cuts that the new Republican majorities will seek to impose on the \$500 billion of discretionary spending controlled by Congress, one-seventh of which goes on science and technology. But biomedical science could be on a collision course with its new paymasters on other fronts. For example, new legislation is likely to ban the type of research on human embryos that the National Institutes of Health (NIH) have only recently begun taking steps to permit.

The hit list of research programmes in the manifesto of Republican Representatives, *Contract with America* — whose mock budget would abolish university research overhead payments, halve spending on several energy programmes, scrap the National Geological Survey and freeze the budget of the National Science Foundation — is described by all sides as almost meaningless, having been drawn up almost at random by committee staff members.

Budgets are expected to fall through steady attrition, rather than by the kind of sudden attacks proposed in the A-Z Spending Bill that the Republicans backed earlier this year as the minority party. "It is more likely to be a stalemate than a turkey-shoot," says Al Teich, director of science and policy programmes at the American Association for the Advancement of Science. He argues that the Democratic minority in the Congress will still be strong enough to exercise a strong influence on budget priorities.

With 47 seats in the 100-strong Senate, for example, where 40 senators can effectively block new legislation, the former majority party is well positioned to obstruct anything it does not like. And the president can still veto budget bills, allowing spending to continue at the previous year's levels. As a result, the new majority party will be able to orchestrate change only if it goes some way to meet the minority Democrats.

But the need to compromise on budgets and legislation means that Republicans are expected to make vigorous use of the unfettered oversight and investigation powers that they won last week with control of



Hatfield (left) and Specter: powerful voices on the Senate's new budget committees (see below).

every House and Senate committee.

Science is likely to get caught up in the consequent rush of supervision. Some senior academics, for example, fear a wave of investigations designed to prove that science-funding agencies and universities are wasting public money.

The shape of the battles ahead is already fairly clear. Thus, although the \$11-billion annual budget of the NIH seems safe from attack, the main danger facing the agency is

that it may once again become a political football, with Harold Varmus, its director, caught between the Clinton administration and the Congress on the various emotive issues surrounding reproductive research.

Officials at the National Science Foundation expect efforts to direct its \$3.5-billion programme towards 'strategic goals' will continue, even though their architect, Barbara Mikulski (Democrat, Maryland), will no longer be chairing the appropriations panel that oversees the agency (see below). But Republicans may use the structure set up by Mikulski to change the goals, with greater emphasis for example on national security and less on protecting the natural environment.

The vast defence research budget, which Clinton had pledged to cut to pay for expansion of civil research, will be staunchly defended by the new majority party. And at the Department of Energy, Republicans will try to swing the pendulum back from environmental clean-up towards nuclear weapons research.

Indeed, with Pete Domenici (Republi-▶

... while new faces jostle for power

Washington. Scientific interest groups in Washington have been reassured by an array of familiar faces, each set to assume crucial committee chairmanships, that the dramatic power shift in US politics from Democrats to Republicans following last week's elections is unlikely to have a significant impact on funding for research.

But the ability of individuals such as Senator Mark Hatfield (Republican, Oregon) and Senator Pete Domenici (Republican, New Mexico) — likely to chair the Senate Appropriations and Budget committees respectively — to defend science against the wave of fervent budget-cutters just elected to Congress will be sorely tested in the months ahead.

Agency chiefs and administration officials may come and go, but those who set the priorities for the US government's \$73-billion science and technology programme are the chairs of congressional committees, all of whom will now be replaced. Such titans as Representative John Dingell (Democrat, Michigan), Senator Bennett Johnston (Democrat, Louisiana) and Senator Robert Byrd (Democrat, West Virginia) have each, as the *Wall Street Journal* put it, gone from power-broker to pumpkin in a day. Who will replace them?

In the Senate, at least, decorum will prevail, and committee chairs are likely to

be appointed by seniority. The liberal Hatfield — a staunch supporter of biomedical research, and an enthusiastic dispenser of earmarked funds to academic institutions — is therefore almost certain to take the chair of the Appropriations committee.

Hatfield is also expected to take over the old fiefdom of Bennett Johnston as chair of the energy appropriations subcommittee. This would leave Arlen Specter (Republican, Pennsylvania), another practitioner of pork-barrel politics and a solid supporter of biomedical research, to assume the chair of the labor, health and education appropriations subcommittee, which funds the National Institutes of Health (NIH).

The third appropriations subcommittee with major scientific jurisdiction is the veterans affairs, housing and urban development and independent agencies (VA-HUD), which funds the National Science Foundation (NSF) and the National Aeronautics and Space Administration (NASA).

This is likely to be forsaken for grander things by its more senior Republican members, including Senator Phil Gramm (Texas). As a result, the subcommittee may fall to Chris Bond (Missouri), whose interest in space and technology springs from the importance of the aerospace giant McDonnell Douglas to St Louis.

As far as Senate authorization commit-▶

►can, New Mexico) — sometimes known as the Senator for Los Alamos — poised to chair the Senate Budget committee, the prospects for Los Alamos and Sandia nuclear weapons laboratories look bright.

The Commerce Department is likely to bear the brunt of Republican hostility to Clinton's technology policy. Almost every observer has singled out the fast-growing programmes of the National Institute of Standards and Technology as a priority target for Republican budget hawks.

In contrast, the National Aeronautics and Space Administration (NASA) may be the least affected by the power shift in Congress. Its strongest supporters are Republicans, but neither the money nor the political impetus exists to reverse the cuts planned in the agency's budget over the next five years.

Furthermore, if the prospects for federally funded technology prospects look gloomy, those for basic science — which Republicans have often favoured — may not be so bad.

Burt Richter, director of the Stanford Linear Accelerator Center (SLAC) in California and president of the American Physical Society, is one of those who expects there to be more emphasis on basic research. "The biggest pressure is going to fall on programmes that involve government with industry," he says. **Colin Macilwain**

►tees go — those that write laws — the Labor and Human Resources Committee, previously the power-base of Senator Edward Kennedy (Democrat, Massachusetts), will go to the socially liberal Nancy Kassebaum (Republican, Kansas).

Energy and Natural Resources will probably be chaired by Frank Murkowski (Alaska), an oil and gas industry supporter. And the hitherto rather inactive science, technology and space subcommittee of the Commerce Committee could well be chaired by Conrad Burns (Montana), who shares vice-president Al Gore's enthusiasm for ensuring access-for-all on the information superhighway.

Changes in the House of Representatives are less predictable as the new speaker, Newt Gingrich (Republican, Georgia) and his right-wing supporters will not necessarily allow senior, liberal republicans to assume influential chairs automatically.

At the Appropriations Committee, for example conservative knives are already out for ranking member Joseph McDade (Pennsylvania), who is under indictment for alleged bribery but is still technically entitled to assume the chair.

Next in line is another centrist, John Myers (Indiana), whose fate could be something of a bellwether. If he is ousted by the right when the Republican caucus meets in early December to allocate the chairmanships, scientists' hopes of business as usual are unlikely to be fulfilled over the next two years.

On seniority grounds, John Myers would

Russian academy seeks a wider role in military research

Moscow. In a move that has apparently won the backing of the Russian government but dismayed many outside observers, the Russian Academy of Sciences (RAS) has announced plans to set up a new constituent body to adopt responsibility for all government-supported applied science, in both the military and civilian sectors.

The surprise move, announced last month after a series of secret meetings, was proposed by Evgeny Velikhov, the academy's vice-president. There is confusion about the name of the new body — on some documents it is called the Russian Science and Technology Academy (RSTA) and on others the Russian Technical Academy.

According to some sources in Moscow, an official order setting up such an academy has already been signed by the first Vice-Premier of Russia, Oleg Soskovets. It will not be independent but will be an arm of the academy.

Such was the secrecy under which the plan was being prepared that neither Goskomimushchestvo (the State Property Committee), nor the Ministry of Sciences

was consulted, even though legally they should have been.

According to the draft charter of the RSTA, its aim is to unite research institutes, scientific centres, research and production associations, factories, laboratories and other organizations that currently fall within the jurisdiction of the State Committee of Defence Industries, the Ministry of Atomic Energy. It will also include other ministries and agencies involved in high technology research and development.

The first 200 academicians, forming the nucleus of the new academy, will be appointed. Another 200 will be elected at a later date in a system similar to that used in the RAS. The structure proposed for the RSTA is similar to that of RAS. But the RAS has not so far been able to solve two problems, namely the legal status of the new organization and its rights to property. The draft charter does not say whether the RSTA will be a state enterprise or a public association. Nor is it explicit about property rights.

But the RSTA will differ from RAS in at least one respect. The RAS is free of any supervision, but the activities of the new academy will be controlled by a board, headed by the first vice-premier of Russia. Other board members will include the president of the RAS, the deputy minister of economics, the minister of atomic energy, the minister of defence, and the chairman of the State Committee of Defence Industries. The chairman of the Federal Counterintelligence Service (formerly KGB), and the mayor of Moscow will also be members.

Vsevolod Medvedev, a member of the RAS presidium, told a press conference in Moscow that the creation of the new academy was the only way to save the Russian military research and development complex from collapse. After the break-up of the former Soviet Union and its ministries, applied research in Russia, starved of financial support and often ineffective, went into rapid decline. Some research institutes were closed down, while others were deprived of their property and research equipment, or began to function merely as commercial real estate.

Yet many doubt the wisdom of the academy's move. Andrei Fonotov, the deputy ministry of science, says that the ministry's efforts to provide selective support to well-performing enterprises is starting to show results — a conclusion confirmed at a meeting with foreign science policy experts last month in St Petersburg.

But Fonotov warns that the situation remains fragile, and that the planned "supermonopolization" of applied science may kill any chance of further progress by increasing bureaucratic controls and reducing flexibility. **Vladimir Pokrovsky**

take the energy subcommittee at Appropriations, John Porter (Illinois), a long-time friend of NIH, the labour, health and education subcommittee, and Jerry Lewis (California) would get VA-HUD. But Lewis is an old enemy of Gingrich's, and none of these positions is assured until after the caucus meets.

The same uncertainty hangs over the House authorization committees, with the added complication that jurisdictions may be changed to streamline the legislative process and cut staff. But one certainty is that Thomas Bliley (Virginia), the tobacco industry's most outspoken congressional supporter, will take over the health and environment subcommittee of Energy and Commerce.

The full Energy and Commerce Committee — which John Dingell built into a formidable power centre over decades — may well be broken up in changes of jurisdiction that could also affect the Science, Space and Technology Committee, previously chaired by George Brown (Democrat, California).

A strengthened Science, Space and Technology committee could then be chaired by one of three possible candidates: Robert Walker (Pennsylvania), if he fails in a bid to become Republican whip; James Sensenbrenner (Wisconsin) if he fails to get the Judiciary Committee; or Sherwood Boehlert (New York), who actually wants the job of heading the only committee on Capitol Hill with a deep and sustained interest in science. **C. M.**

Researchers face delay in supplies of RU486

Washington. The politics of abortion in the United States is holding up clinical and pre-clinical research on RU486, the so-called 'abortion pill', which also promises many other biomedical applications in treatments ranging from breast cancer to brain tumours.

Earlier this year, faced with the heat surrounding the abortion debate, Roussel-Uclaf, the manufacturers of the drug, handed over the US patent rights to the Population Council in New York. The council, a non-profit group, has agreed to find a manufacturer and distributor in the United States and make the required New Drug Application to the Food and Drug Administration.

But the council — which has yet to name a manufacturer — will not have a stock of the drug for at least a year. Meanwhile, Roussel-Uclaf has stopped supplying the drug to new investigators. Supplies to existing trials, such as those for a type of brain tumour and the Population Council's abortion trials, will continue.

Wayne Bardin, director of the council's Center for Biomedical Research, has heard from about 20 investigators who have been affected by supply problems; some began writing to him in September to ask for supplies of the drug at the suggestion of Roussel-Uclaf. Yet despite such demand, Andre Ulmann, director of research and development in endocrinology at Roussel-Uclaf, says the company will not be supplying RU486 to new investigators because of its contract with the Population Council.

One possible solution suggested by Bardin is for the Population Council to

distribute the drug for Roussel-Uclaf until stocks are manufactured in the United States.

The current situation is the latest manifestation of the problems faced by US scientists interested in research with RU486. RU486 (mifepristone), is an antiprogesterin, and it is important because although it binds progesterone receptors it has little progesterone-like activity. It prevents the progesterone effects needed to establish pregnancy, thereby causing abortion and it is this property that has created the political pressure to keep it out of the US.

But, as an antiprogesterin, RU486 has much wider possible applications. In 1993, the US Institute of Medicine (IOM) released a report on the clinical applications of antiprogesterins, describing them as having "a significant potential in human health". The institute cited the need to investigate the potential use of antiprogesterins as a therapy for meningioma (a tumour of the membrane surrounding the brain), endometriosis, breast cancer and uterine leiomyomas (fibroids).

Mary Lake Polan, chair of the department of obstetrics and gynaecology at Stanford University in California, and a member of the committee that prepared the IOM report, says that in the current climate it is difficult to blame Roussel-Uclaf for wanting to keep RU486 at arms length.

Polan describes the IOM report as "an attempt to put RU486 back into the realm of science". But the move does not seem to

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Demonstrations have made companies nervous

have succeeded. Several scientists failed to return repeated telephone calls from *Nature*. "You may have noticed [anti-abortionists are] shooting people," said one who did. "It's no longer a science issue, and it's hard to predict what people driven by a conviction of their own moral superiority will do."

One scientist who has repeatedly spoken out on the potential value of RU486 is Sam Yen, professor of reproductive medicine at the University of California, San Diego. "The emerging evidence is that this powerful new drug might have utility in the brain and the reproductive system," says Yen. "But these potential benefits are being deterred by politics." His own work was halted 18 months ago when he was caught up in a battle between Roussel-Uclaf and its German parent company Hoechst over whether to continue producing RU486. But he too is reluctant to blame the company when "their property might be burnt".

Yen has recently received a supply of RU486 through an informal international group of scientists. This source, however, is unable to meet all demands for the drug.

Canadian and European researchers are already evaluating RU486 for treating breast cancers. But the US National Cancer Institute has decided not to pursue research with this molecule. "We watch the literature, but for now we have many other promising therapies," says Michael Christian, head of the National Cancer Institute's developmental chemotherapy section.

Another part of the US National Institutes of Health, the National Institute of Child Health and Human Development, has sponsored work at the Research Triangle Institute in North Carolina to develop an antiprogesterin. Ed Cook, the head of the project, says it has a candidate compound but that it is a long way from being available for research with patients.

Roussel-Uclaf continues to face controversy, with both external and (it is said) internal pressures to abandon RU486 completely. Ulmann admits there are rumours that the company is pulling out of work on antiprogesterins, but says that no such decision has been made.

Helen Gavaghan

Changes urged in NIH peer review process

Washington. The US National Institutes of Health (NIH) are being urged to appoint senior scientists to act as 'at large' members of its peer review committees.

The members would provide "a new measure of wisdom, perspective and continuity in the review process" says a report prepared by staff of NIH and Keith Yamamoto, head of the department of biochemistry and biophysics at the University of California, San Francisco and based on a meeting between NIH officials and researchers in September.

Since the arrival of Harold Varmus as NIH director, peer review — a key element in the distribution of the \$8 billion that the NIH spends annually on extramural research — has itself come under close scrutiny. Yamamoto is an active participant in debates on biomedical research policy and took part in an *ad hoc* meeting on peer review chaired by Varmus at NIH earlier this year (see *Nature* 369, 269; 1994).

The September meeting, which Yamamoto chaired, brought together for

the first time NIH administrators from the review section responsible for the biological and physiological sciences with scientists who receive extramural grants.

The report recommends:

- An 'at large' member of review committees who would observe all study sections in a particular area of science, informing each of relevant developments in an area covered by a closely associated study section;

- A broadening of expertise on study sections to help them to keep up with rapidly developing areas of research;

- Continuing scientific education for the NIH's scientific review administrators;

- A relaxation of the rules defining the distribution of reviewers according to geographical areas or institutions, in order to ensure the best reviewers for the job can be selected;

- Further efforts to clarify the review process for applicants; and

- A continuing review of the scope and quality of peer review from different study sections.

H. G.

Gene therapy network sets industrial mould

Paris. The French/US pharmaceutical company Rhône-Poulenc Rorer (RPR) has announced an ambitious plan to create a new form of industrial organization for commercializing gene therapy through a coordinated network of academic research groups and biotechnology companies.

The company's plan is to use its network to access the many different technologies needed for such therapy. Biotechnology analysts have enthusiastically welcomed the move, arguing that a network approach may be more appropriate for gene therapy than either the traditional approach to drug research in big companies, or the US model of biotechnology start-ups.

Last year RPR took a 38 per cent stake in Applied Immune Sciences, a California gene-therapy company. It followed this by regrouping its biotechnology activities into a single division focused on gene therapy, Biotech (see *Nature* 369, 92; 1994).

The new division, Gencell, will enlarge Biotech to involve a consortium of 14 academic groups and biotechnology companies. Thierry Soursac, senior vice-president of RPR and head of Gencell, says the idea is to "get one foot" in each of the technologies needed to commercialize gene therapy.

"The winners in gene therapy will not be those with the genes, but those who can deliver the product," says Peter Smith of the London stockbrokers James Capel. "It's different to a pill in the bottle, and more like the diagnostics industry."

Rachel Leheny, an analyst at Hambrecht & Quist in New York, says that while many companies are pushing ahead in cell therapy, genomics and gene therapy, RPR appears to be the first to try to "straddle" all three areas. Most of the present gene-therapy and genomics partnerships are "missing a part of the puzzle", she says; in contrast, RPR has taken strategic alliances a step further "by going for a coordinated approach".

"If Gencell has all the parts to deliver genes, it will also be an attractive partner for genomic companies," says Smith.

It is also spreading its net wide. In gene sequencing, for example, Gencell has formed a Cooperative Research and Development Agreement (CRADA) with the Lawrence Berkeley Human Genome Center in California to access genes for the treatment of diabetes, obesity, cardiovascular diseases and nervous disorders.

Gencell includes links (through RPR's recent equity stake) to Darwin Molecular, the genome company based in Seattle, Washington, and to the French company Généthron. It also has access to each of the current classes of vector through the Université Louis Pasteur and the biotechnology company Transgène — both based in Strasbourg, France — and groups at the Centre National de la Recherche Scientifique

(CNRS), Introgen Therapeutics and Applied Immune Sciences.

In practice, scientists from the members of the consortium will sit on programme boards within Gencell to develop therapeutic projects. Royalties will be agreed in advance; for example, ownership of a gene will bring royalties of 6 to 10 per cent on sales, and a vector will bring 5 per cent.

Soursac argues that "it's a joke that large drug companies think they can automatically handle clinical trials in gene therapy the same way they do traditional drugs". He says that drugs come through trials either "dead or alive", but genes will go backward and forwards with progressive modifications, for example, to vectors or regulatory sequences. "Speed and success will depend on having the players and competencies close to hand," he says.

But while analysts are enthusiastic about RPR's general approach, they are more cautious about its own chances of success. One concern is that the arrangement is 'loose', in that RPR is only financing research programmes and has held back from the big outlay needed to buy equity stakes or obtain exclusive deals. "They are getting young companies desperate for cash," says one critic. "Medium-sized companies are talking to RPR, but they are not going to settle for only US\$5 million up-front."

But Soursac argues that gene therapy is a "moving target", and that it is "too soon and

too risky" to take majority stakes in companies. "I'm not paying more than \$5 million up-front, plus milestone payments," he says. "When the time comes I will decide whether to grab or not." Soursac will seek new partners and eventually take majority stakes in members of the network "at market prices".

Soursac also says, for example, that the Lawrence Berkeley Human Genome Center could have found a more attractive financial deal than his, but that its director, Edward Rubin, was attracted by the concept of Gencell. "He was more interested in the network, the technology base and the quality of scientists than the money," says Soursac.

Indeed, the magic spell of Bill Gates, chief executive officer of Microsoft and a board member of Darwin Molecular — which is part of Gencell — seems not irrelevant to Gencell's apparent appeal. "The drug industry today is analogous to the micro-computer industry in the early 1970s," says Gates in a statement circulated by Gencell. "We're doing what Bill did when he didn't have the money he has now," says Soursac.

Another concern is that RPR's 'toolbox' may be missing some essential spanners. "They have to make sure their joint ventures are leading edge," says one critic. "I would be more excited about this if I saw more big names." Soursac admits that Gencell may not have all the ingredients. "But we've got enough to cook dinner." **Declan Butler**

Leonardo text yields a record price

London. Leonardo da Vinci's 'Codex Hammer', a 72-page illustrated treatise on pre-modern science, was auctioned at Christie's, New York, last Friday for US\$30.8 million (£19.2 million) — the highest price ever paid for a manuscript.

Most of the book is devoted to Leonardo's original systematic observations and experiments on the properties and nature of water, which he links to speculations about cosmology, astronomy, tidal theory, meteorology, geology, physical geography and palaeontology.

He incorrectly supposes that sunlight reflected from the "waters" of the Moon is the source of moonlight. But he correctly deduces — as shown in a close-up of the new Moon (see right) — that the secondary light of the Moon is a reflection of sunlight from the oceans of the Earth (the Moon's lit area is shaded).

Leonardo also studies how a stream flows round an obstruction, offers practical advice on flood control, dams and canalization and presents engineering designs for the snorkel and submarine.

Compiled between 1506 and 1508, the 'Codex Hammer' is named after its

previous owner, Armand Hammer, the late American oil tycoon and art collector, who bought it in 1980 for £2.42 million from Viscount Coke, eldest son of the sixth Earl of Leicester. Thomas Coke, the first earl, acquired it in Florence in 1717.

The new buyer is computer entrepreneur Bill Gates, chairman of Microsoft Corporation. He intends to place the work, presumably to be renamed the 'Codex Gates' or 'Codex Microsoft' on public display, starting with a tour of Italian museums. **Peter Tallack**



Critics appeased by changes in UK national curriculum

London. Britain's scientific community has, in general, given a warm welcome to the government's announcement last week of plans to streamline the national curriculum. The result will be to reduce both the amount of material that school students are required to learn, and the time spent by teachers in evaluating their pupils.

But several bodies remain concerned that too many 14–16-year-olds will continue to take the controversial 'single award' science curriculum, which is taught in only 10 per cent of the curriculum time.

The revised curriculum was launched by Sir Ron Dearing, chairman of the School Curriculum and Assessment Authority (SCAA) and Mrs Gillian Shephard, the Secretary of State for Education, and followed a four-month consultation period after a draft version of the revisions was published in May.

The slimmed-down curriculum, with three core subjects, English, mathematics and science, covers 10 subjects in a total of 222 pages, compared to 330 in the original. And 1,000 'statements of attainment', heavily criticized by teachers as a 'tick-list' form of assessment, have been cut to 200 broad descriptions.

The changes in the science curriculum are based on 60,000 responses to the consultation document received by SCAA. They include an increase in the amount of chemistry in both the 13–14 and 14–16 age groups, and an overall reduction in the Earth science component.

Pupils at secondary schools (ages 11–16) took integrated courses of 'balanced science' before the introduction of the national curriculum in 1988. But the spread of balanced science, initiated by the national curriculum, has been well received, not least because it has helped to increase the number of girls studying science past the age of 14.

But there has been widespread concern about the 'single science' award for the 14–16 age group, in which all three topics are covered in only 10 per cent of the curriculum time. Many scientific institutions argue that this is insufficient to give pupils the grounding they need to deal with issues in everyday life, and that the 'double science' award should be the minimum requirement (see *Nature* 370, 406; 1994).

"The proposals for double award science go a long way to restoring the balance of the major subjects and we therefore welcome the draft order," says Tony Ashmore, education secretary for the Royal Society of Chemistry. "But we remain concerned that too

many young people might either choose or be forced into taking single award."

The society had hoped for some guidance on which pupils should be allowed to do single science. But the draft order does not provide any clarification. Dearing supports the government's view that "the great majority" of pupils — around 80 per cent — should take double science. But he says that choosing who should do so is a matter of discretion "for the school, the parents and the children".

The changes to the single-science syllabus include the removal of the section on the physiology of green plants to be replaced by more on human nutrition and a "more overt reference" to living organisms in their natural environment.

In addition, the theoretical aspects of

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Lighter load: targets have been reduced

structure and bonding of compounds in the 'materials and their properties' section have been replaced by work on patterns of behaviour and the periodic table.

But the Royal Society is still concerned. "They have revised it [single-award science] quite significantly, but it's still there and we're worried that pupils will still take it," says Robert Rees, education officer for the society.

The Association for Science Education (ASE) also welcomed the revisions to the national curriculum. But it called for a "more considered evaluation of the educational needs of children in the 21st century and the role which science education has in this".

A particular target now is likely to be post-16 education. Dick West, professor of science education at the Open University and a former president of the ASE, points out that the system for 14–16 year olds is "totally decoupled" from the academic curriculum for 16–19 year olds.

West argues that Britain still needs to move to a broader baccalaureate-type system. Such an idea was proposed by the Royal Society in a report in 1991. "Now that the immediate panic over reforming the national curriculum is over, we will be revisiting post-16 education," says Rees.

Maggie Verrall

Maths minus boys means 'better learning for girls'

Boston. Girls-only mathematics classes may soon be taught in a New Hampshire high school in an attempt to improve girls' examination performance. If the proposal, made by Charles Vaughn, a New Hampshire state representative, is adopted by the school board of Portsmouth High School, a girls-only freshman algebra class will be offered next September. The board is due to vote on the issue in January or February.

Vaughn hopes that the initiative will help to narrow or eliminate the "achievement gap" between boys and girls on the Scholastic Assessment Test (SAT), the national examination taken by US high-school students. Studies have shown that boys attract more attention than girls in the classroom, and Vaughn thinks the relative success of boys may be attributable to this unintentional favouritism — a problem that could be avoided in all-girl classes.

Similar programmes already exist in Ventura, California and Presque Isle, Maine. But the idea is far from being universally accepted. Susan Foye, head of the mathematics department at Portsmouth High School, believes that creating a separate class for girls is unnecessary. "Just design classes in a manner that enables everyone, boys and girls, to thrive," Foye suggests.

Catherine Didion, executive director of the Association for Women in Science, based in Washington, DC, is also in favour of trying to improve mixed classes, although she admits that such improvement will take time to implement.

"In the meantime, why can't we teach an all-girl course and see what we learn from that?" asks Didion.

Steve Nadis

AIDS 'czar' favours basic research

Washington. President Bill Clinton has confirmed the appointment of Patsy Fleming as White House AIDS Policy Director. She has been filling the post on an interim basis since Clinton's first 'AIDS czar', Kristine Gebbie, resigned in August.

Fleming was formerly a congressional staffer active on AIDS issues and, more recently, a special assistant to Donna Shalala, the health secretary.

She has promised to help women and young people protect themselves from AIDS, and to work with William Paul, director of the recently strengthened Office of AIDS Research at the National Institutes of Health, in his effort to concentrate on basic research aimed at understanding the nature of the disease.

Colin Macilwain

Patent on PCR enzymes may re-ignite old controversy

London. Controversy over the patent rights to *Taq* polymerase, one of the basic thermostable enzymes used in the polymerase chain reaction (PCR) process, is likely to resurface following a decision by the European Patent Office (EPO) to grant a broad-ranging patent to the Swiss pharmaceutical company Hoffman-La Roche.

The patent will cover all thermostable polymerases with a molecular weight between 86,000 and 90,000. In practice, this means that Hoffman-La Roche — which paid the US biotechnology company Cetus Corporation \$300 million for the rights to both *Taq* and PCR in 1991 — will be in a position to demand royalties from the manufacturers of all such enzymes, regardless of whether they are officially used for PCR.

Some companies such as Stratagene, the manufacturers of the enzyme Pfu (which will be covered by the patent), have already agreed to enter licensing deals with Hoffman-La Roche, which is expected to receive up to 20 per cent of the list price of the enzymes. According to Hoffman-La Roche, negotiations with other manufacturers are under way.

But, given the breadth of the claim — the US patent, issued in January 1990, covers only *Taq* polymerase, and is restricted to its use in PCR — as well as the high financial stakes involved in a technology that has become a staple tool of most molecular biology research, at least one company, Promega Corporation of Madison, Wisconsin, is already planning to contest the patent.

Promega is currently being sued by Roche in the United States for breaching a licence agreement to sell *Taq* for non-PCR purposes, and is in turn challenging Roche's US patent on *Taq*, primarily on the grounds that the enzyme had previously been purified by Russian scientists (see *Nature* 364, 2; 1993).

Other companies are being more cautious. Ira Schildkraut, research director of New England Biolabs, whose wide-selling products VENT and Deep VENT would also be covered by Roche's new patent, merely says that the company is keeping "a close eye" on the situation.

Thermostable polymerases are valuable because they can be used continuously through successive heating cycles used to amplify a sequence of nucleotides in PCR. The EPO initially objected to the Roche application on the grounds that the Russian researchers had published details of a similar thermostable enzyme extracted from the same organism, the bacterium *Thermus aquaticus*. Roche argued that the enzyme purified by Cetus had a considerably greater accuracy in reproducing nucleotide sequences than the Russian enzyme. This claim appears to have been sufficient to persuade

the EPO of the genuine novelty of the Cetus discovery.

News of the preliminary decision was passed to the company by the EPO patent examiner in August. The patent is due to take effect early in the New Year, permitting Roche to take out legal proceedings against those they consider to be infringing it.

Roche officials say they intend to pursue an "active licensing policy" for thermostable enzymes such as *Taq* "in an effort to make the PCR technology as widely available as possible". The forthcoming patent, they say "will put Roche in a position to continue to actively protect its own interests, as well as those of its licensees". In practice, this is likely to mean that Roche will now increase its efforts to negotiate licensing deals (based on royalty payments) with all other manufacturers of thermostable polymerases.

In theory, the company could also take action against scientists in research laboratories who produce their own *Taq*. But the company says that is unlikely. "It is not our strategy to resolve disputes at the level of researchers," says the company's PCR licensing manager, Agnieszka Junosza-Jankowski.

Many research organizations that use large quantities of *Taq* say that, although concerned at the increased costs that will inevitably follow the granting of the patent, they intend to pay up because Roche is legally entitled to claim such royalties.

But some scientists point out that *Thermus aquaticus* is a naturally occurring organism, and others fear that the breadth of the patent might discourage the efforts of other research groups to develop more efficient thermostable enzymes.

Such arguments are likely to be repeated in court if the legal challenge materializes. Although two challenges to the patent application on behalf of unnamed companies have been rejected by the EPO, some (such as Promega) feel that the battle is far from lost — and promise to produce fresh arguments against the patent.

Roche says it is confident it can defend the patent, both in the United States and, if necessary, in Europe. It points out that, in issuing their initial patents, both the US patent office and the EPO have already rejected claims that the Russian scientists got there first.

But others point out that, just because an argument against a patent is rejected by one patent examiner, it does not mean that this argument cannot be used again in a legal challenge to the patent. "It will be interesting to see how this one develops," says one patent attorney who has been closely involved in the issue. **David Dickson**

Insurance company to back out of some climate-linked risks

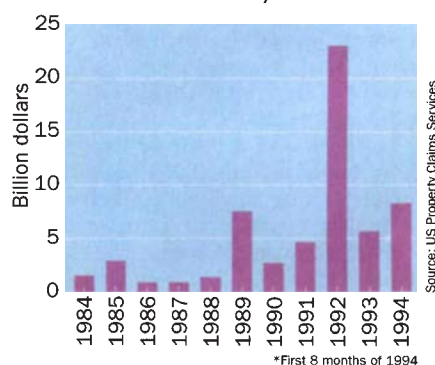
Munich. Munich Re, the world's largest reinsurance company, has called on governments to meet their commitments to stabilize greenhouse gas emissions in line with the world climate convention, signed in Rio de Janeiro in 1992, because of threats from the impact of climate change.

A company spokesman, Wolf Otto Bauer, said last week that the company is "convinced that the trend towards more frequent and more severe natural disasters will continue in the future", and that it intends to reduce some of its high-risk activities in areas related to climate change.

Both statements reflect growing concern among insurance companies that global warming may be responsible for a recent sharp increase in insurance claims resulting from natural disasters (see chart), which have resulted in several bankruptcies in the industry.

They come shortly after the publication of an independent report on the long-term financial risks of climate change to the oil

Insured losses from major natural disasters in the United States, 1984-1994*



and gas industries commissioned by Greenpeace from the independent Delphi Group of financial consultants.

The report was written by Mark Mansley, former chief analyst with Chase Investment Bank, and is the first study of the impact of climate change on capital markets. It concludes that investors should avoid putting too much money into oil and gas companies because measures to curb carbon-dioxide emissions, which it predicts will be introduced within the decade, will turn such businesses into poor investments.

In addition to Munich Re, a number of other major insurance and reinsurance companies are also becoming involved in the global warming debate. But they are not yet committing themselves to specific policy changes, despite pressure from environmental groups to take more direct steps to help shift energy policy away from fossil fuels.

"It has been bewildering to me to find▶

Tension at the top in South African science

Cape Town. Shortly after last May's general election in South Africa, President Nelson Mandela established a Ministry of Arts, Culture, Science and Technology, and appointed Dr Ben Ngubane, one of three cabinet members belonging to Mangosothu Buthelezi's Inkatha Freedom Party, as its minister.

At the same time, Mandela chose his estranged wife Winnie, the chairwoman of the African National Congress (ANC)'s Women's League, to be Ngubane's deputy minister. Six months later, the two appear to be going through a tempestuous honeymoon. Whereas Ngubane appears cautious in his desire for change, Mrs Mandela has had little hesitation in expressing her frustration with the inertia of government bureaucracy.

As a result, the two appear sometimes to be out of step. In May, for example, Mrs Mandela announced the establishment of an advisory committee for the ministry; Ngubane announced the establishment of a separate committee in June. It was only after intervention by the deputy-president, Thabo Mbeki, that a 'definitive' committee was finally constituted in August to replace the old Scientific Advisory Council (SAC), whose term of office had lapsed.

The new committee is chaired jointly by Friedel Sellschop, a nuclear physicist and deputy vice-chancellor for research at the University of the Witwatersrand, and Fatima Meer, a sociologist at the University of Natal. The minister and his deputy each recommended the appointment of four members to this committee, two for arts and culture, and two for science and technology.

The advisory committee will decide how

to divide the science vote, currently totalling R886 million (US\$253 million), among the seven research councils (including the newly created Council for Geosciences) and the Bureau of Standards.

The value of the vote has dropped by 27 per cent in real terms since 1987. But Ngubane is confident that it will increase next year, pointing out that, under the terms of the Reconstruction and Development Programme (RDP), the government is committed to now increasing expenditure on research and development in South Africa.

He is less optimistic that funds previously allocated to military research will be redirected towards the civil sector, saying that it is "too early" for such a decision. In addition to the science vote, the government provides a R509-million subsidy to the Atomic Energy Corporation and an undisclosed subsidy, which is almost certainly far larger, to the state-owned armaments industry.

Academics have welcomed the appointment of Sellschop and Meer as co-chairs of the advisory committee. Sellschop, in particular, is known to favour a larger portion of the science vote being allocated to agency funding (see *Nature* 362, 487; 1993).

As before, the advisory committee's recommendations will be forwarded to a cabinet committee, which makes the final allocation of funds. This committee is made up of the ministers of Arts, Culture, Science and Technology, Trade and Industry, Mineral and Energy Affairs, Agriculture, and Health, and is chaired by Ngubane.

ANC ministers are in a minority on this committee. But it may be extended to include the minister responsible for the RDP,

the ANC minister Jay Naidoo.

Ngubane says the most important change is the way in which funds will be allocated to the science councils, increasing the weight given to national needs and priorities in deciding such allocations.

The government's decision has prompted the research councils hurriedly to redraft their existing and planned activities in terms of the new programme's stated aims. Whether funding for basic research will be affected depends heavily on whether the minister is able to persuade the Treasury to increase the value of the science vote.

Ngubane says that he recognizes the role of the Plenary of the Science and Technology Initiative (see *Nature* 367, 211; 1994) as a national forum on science and technology, but feels it should expand its representation. He has invited the body to commission a foresight study on science and technology within the framework of the RDP.

Ngubane and Mrs Mandela seem to agree on one matter at least: that the RDP should be treated as a holy grail. But they appear to differ in their evaluation of what needs to be done to further its cause. Ngubane is inherently cautious of change. But Mrs Mandela has no such qualms, singling out the Council for Scientific and Industrial Research and the Human Sciences Research Council for criticism in a recent debate on the ministry's budget in parliament. In particular, Mrs Mandela pointed out that the staff of both are "overwhelmingly white and male". She said that this was "not surprising", given that it represented the "direction in which the Nationalists operated their 'affirmative action' policy".

But her harshest criticism was reserved for her own ministry. She claimed that, although the department's staff is trying to accommodate the demands of the RDP, "if truth be told, few have the stomach for it". Ngubane's response to this is that his deputy "was making a political speech, which she is perfectly entitled to do". **Michael Cherry**



Ngubane: cautious of big changes.

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Mandela: pushing against inertia.

► that people in the financial industry in New York, London, Washington and Tokyo are all saying the same thing — that climate change threatens the survival of their businesses — and yet no-one has yet responded to it as a risk," says Jeremy Leggett, scientific director of Greenpeace's climate campaign.

Leggett wants insurance companies to switch their investments from fossil fuel businesses, which he says undermine the insurance companies' own market security, to 'greener' programmes such as solar energy and energy-efficient housing. The Delphi report endorses this position, stating that "the alternative energy industry offers greater growth potential than the carbon-fuel industry."

Leggett also wants representatives of the financial sector at the negotiations of the Intergovernmental Panel on Climate Change (IPCC), pointing out that "the oil, coal and auto industries have been represented from day one."

Despite lack of direct representation, Andrew Dlugolecki, from the UK insurance company General Accident, is working with IPCC, which publishes regular scientific assessments of climate change, on a chapter on global warming and the financial sector for a forthcoming report, due in 1995.

He says that insurance companies are taking the issue extremely seriously, but that most are reserving judgement. "A change of policy is probably premature", he says, although adding that he believes

that insurance companies may eventually have to adopt the line suggested by Greenpeace.

Dlugolecki is critical of the lack of interest banks have shown up to now in global warming, despite the fact that many of their long-term investments and loans — such as building projects — are highly vulnerable to natural disasters such as storms, floods and hurricanes.

But banks are beginning to wake up to the dangers. A group of 45 banks from around the world met in Geneva last month for a meeting organized by the United Nations on banks and the environment, and British bankers will hold a joint seminar with Greenpeace on the subject next week.

Alison Abbott

Monsanto defends itself

SIR — The main thesis of the Commentary by Millstone *et al.*¹ is that Monsanto has suppressed information on the real change in somatic cell content (SCC) of milk from bovine somatotropin (bST)-supplemented cows and that this has public-health implications. The authors contend that they were justified in trying to publish an analysis of Monsanto's data without permission from the scientists who gathered the data.

Science is a self-correcting institution striving to find truth based on published data. The truth in this case is that the impact of bST on SCC of cow's milk has been widely published²⁻⁸ by university, government and corporate scientists. Moore and Hutchinson⁴ reported that 18 of 20 published studies measured a slight decrease or no change in SCC of milk from bST-supplemented cattle and two showed a slight increase. Thomas *et al.*⁷ summarized data from 15 US commercial herds demonstrating no effect of bST on SCC of milk. Monsallier³ reported that SCC of milk was not affected from 19 French commercial herds using Monsanto's bST formulation and three French research trials using Eli Lilly's bST formulation. McClary *et al.*² published data on six research studies using Eli Lilly's bST formulation and reported a slight increase in SCC of milk from bST-supplemented cows. The papers cited represent data on more than 5,000 dairy cows from 77 individual studies in peer-reviewed articles, hardly a suppression of the facts. This literature was not mentioned in ref. 1.

The SCC data from Monsanto studies were submitted to the Committee for Veterinary Medicinal Products (CVMP) of the European Union as well as the Center for Veterinary Medicine (CVM) of the Food and Drug Administration (FDA) in the United States. The CVM also conducted a public hearing on mastitis incidence in bST-supplemented cows (31 March 1993) where the Monsanto data were again reviewed by the FDA. The FDA and the CVMP are the appropriate regulatory authorities for evaluation of human and animal safety. These organizations have carried out their own analysis and review of the data, and both concluded that bST is safe for use in lactating dairy cows to increase milk production. Further, the entire dataset from all Monsanto studies is published in ref. 8. All the individual studies were published separately or, in the case of the clinical trials, as a group in several journals, and these are referenced in ref. 8. In our view, these

data and the published conclusions of the appropriate regulatory bodies do not support a public-health risk from milk derived from bST-supplemented cows.

Robert J. Collier

Douglas L. Hard

The Agricultural Group,

Monsanto,

700 Chesterfield Parkway North,

St Louis, Missouri 63198, USA

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A matter of degree

SIR — In a News item about Dutch proposals to introduce short degrees (*Nature* **371**, 95; 1994), it is stated that this will be a first for mainland Europe. Some years ago, however, Denmark took this step, although without the draconian financial cuts envisaged by the Dutch.

Until then, the first degree in Denmark was equivalent to a master's, taking between five and seven years, several of which were spent in research. The then Minister of Education, Bertil Haarder, introduced the bachelor three-year degree despite protests from academics, industrialists and schoolteachers. Opponents claimed that those with bachelors' degrees would be unemployable, and this has largely proved true. There has been so much resistance by the universities that very few students take this degree if they can avoid it. The Dutch government should think again about this proposal, which may become an embarrassment, as it did here in Denmark.

Dieter Britz

Department of Chemistry,

Århus University,

DK-8000 Århus C, Denmark

Wrong policy?

SIR — In a recent leading article on genome databases (*Nature* **371**, 363; 1994) you comment: "The fear that this asymmetry [between the contributions to and uses of databases by industrial and academic scientists] would become a problem is partly why *Nature* resolved . . . not to make the submission of sequences

to the databanks . . . a condition of publication. It is naive of the research community not to have shared that caution."

This statement is a self-serving and somewhat pompous defence of an unusual (compared with other first-rate scientific journals) policy on *Nature's* part that only exacerbates the problem. As a scientific community, we must strive to establish a balance between competition and cooperation that best fosters progress. The pressures leading to competitive sequestration of data are intrinsic and strong within both academic institutions and industry. One would hope that the major journals and funding agencies would, for the greater benefit of the whole, strive to provide counterbalancing forces that favour cooperation and data sharing. Most do. Obviously journals that do not participate in this convention gain individual advantage by obtaining manuscripts from less altruistic members of the community. But, this is at the expense of the community as a whole.

David Shalloway

Section of Biochemistry,

Molecular and Cell Biology,

Cornell University,

Ithaca, New York 14853, USA

Blanket coverage

SIR — The recent article "HGS seeks exclusive option on all patents using its cDNA sequences" (*Nature* **371**, 463; 1994) has stimulated us to announce the formation of our new institute, the Random Genome Research Institute (RGRI). The efforts of RGRI will soon make the interesting battle of the pharmaceutical giants over cDNA sequences irrelevant. We have gone to considerable trouble to generate all possible cDNA sequences of 2,000 bases in length. Our extensive sequence generating project will give us access to all patentable cDNA before conventional sequencing can be done. We do not intend to infringe the patent rights of those investigators who can prove the possession of sequence data before our public announcement. Sequences that contain segments from our database will also be covered in our patent application. After exercising our exclusive option for any sequence, we will gladly discuss terms for collaborations and licences on any patented sequences. Profits from RGRI will be used to fund new efforts in biological research to understand how our sequences can be combined to create organisms.

Kevin Ainger

Youhe Gao

Yilong Sun

RGRI, c/o Department of Biochemistry,
University of Connecticut Health Center,
Farmington,
Connecticut 06030-3305, USA

Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only.

Endangered telescopes or species?

Bruce Walsh, Roger Angel & Peter Strittmatter*

The planned international observatory at Mount Graham, Arizona, provides a model of how environmental concerns should be met in a project of this size. But it remains vulnerable to irresponsible "activists".

THE Mount Graham International Observatory project is in the news again as litigation once more delays progress, this time on the Large Binocular Telescope (LBT). This telescope, like two others that have already been completed, is being built under provisions made in a 6-year-old "Biological Opinion" issued by Michael Spear, director of the US Fish and Wildlife Service in compliance with the Endangered Species Act. The new lawsuit, brought by "environmentalists" against the Forest Service, argues that the agency had no authority to revise the LBT site by a few hundred yards to a place where it would have less effect on the endangered red squirrel. This bizarre action suggests motives other than protection of an endangered species. In fact, the situation at Mount Graham illustrates some of the more general problems facing science and public policy today.

The Mount Graham observatory is the first to be built in this era of increased environmental awareness. After 10 years of evaluations and biological study, and in collaboration with international partners, two telescopes have been built and are generally viewed as models of technical innovation, compactness and environmental sensitivity. Yet there remains a core of opponents, addicted to confrontation, waging an intense campaign to stop telescope construction at any price.

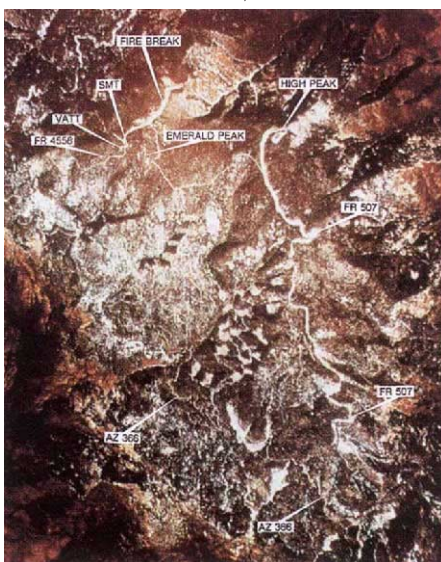
The strategy of these activists is to manipulate environmental laws and to intimidate participating institutions with delays and uncertainty created by lawsuits, demonstrations, sit-ins and mail-ins. Such misguided activism, in this case directed at halting a benign, legitimate project, is eroding public support not only for the environmental organizations involved, but also for important environmental laws.

Selection of the site

For a century, telescopes taking advantage of the clear skies and mountains of the American southwest have been in the forefront of astronomical discovery. Today, however, the long-established observatories are either being hurt by light pollution or are too low in altitude for the new observational opportunities at infrared and submillimetre wavelengths. Thus, in the early 1980s, the University of Arizona and the Smithsonian Institution

began a search for a new world-class telescope site in the continental United States. Mount Graham was singled out as an outstanding candidate on the basis of clear, dark skies, low precipitable water vapour, and accessibility. At 3,200-m elevation, it is the highest of the "sky island" mountains of the Arizona-Sonora desert, and has excellent image-sharpness characteristics.

On the other hand, the features that



Aerial photograph taken in early 1991 of roughly 8,000 acres of Mount Graham, showing the clearings for the VATT (0.3 acres), the SMT (0.4 acres) and the 2-mile observatory access road, FR 4556. The Emerald Peak site for the LBT (1.2 acres) was cleared in 1993. Snow cover delineates areas of higher elevation and of pre-observatory impacts. Noteworthy are the state highway (AZ366), Forest Service road FR507 (and fire break), the fire break at Emerald Peak and the extensive logging activity between AZ 366, FR 507 and FR 4556. (White areas have been cleared, dark areas are still forested.)

make Mount Graham and other Arizona sky-island sites so desirable for astronomy also excite great biological interest, as they mark the transition from boreal to madrean ecosystems. A prime consideration in selecting Mount Graham was the already high level of human activity on the mountain, which makes the incremental impact of the observatory very small. The aerial photograph should make this abundantly clear. Mount Graham has a paved, 30-mile 2-lane access road, has been heavily logged, both by tree selection and by clear cutting (more than 5,000 acres),

and hosts transmitter stations, 93 summer homes, an artificial lake and 250,000 visitors a year. It is not a pristine mountain.

The request by astronomers to place telescopes on Mount Graham in 1984 led the US Forest Service to undertake a 4-year evaluation of biological and other environmental impacts. Red squirrels were removed from the hunting list and classified as an endangered species. Timbering activity was stopped. Under the provisions of the Endangered Species Act, a biological opinion allowed for a strictly controlled, phase I, three-telescope observatory, limited to 8.6 acres. Approval was subject to implementation, at observatory cost, of many protective provisions, among them reforestation of 60 acres of road, creation of a red squirrel 'refugium' covering 882 acres of spruce/fir forest, and a squirrel-monitoring programme (six full-time biologists).

Establishment of the observatory

The observatory, foreseen in the 1984 Arizona Wilderness Act, was confirmed in the 1988 Arizona Idaho Conservation Act (AICA) which adopted the results of two environmental impact studies and the biological opinion, and authorized the immediate construction of the first three telescopes. Its purpose was to prevent unnecessary further delay. Two of the first three telescopes have been constructed and are now being commissioned: the Vatican Observatory 1.8-m Lennon telescope and the 10-m Heinrich Hertz Submillimeter Telescope, a joint project of the Max Planck Institute for Radio Astronomy, Germany and the Steward Observatory.

The third telescope, the LBT, is a collaboration between Arizona, Italy (Arcetri) and the Research Corporation and will have two 8.4-m diameter mirrors on a single mounting. Its light-gathering power, equal to an 11.8-m single mirror, will be significantly greater than any other single telescope, and its ability to resolve faint objects will be unrivalled when coupled with new adaptive optics techniques to remove atmospheric aberrations. The LBT will be a unique, very sensitive telescope, gaining much of its special nature from the advanced technology made possible by the excellent logistics of the Mount Graham site. It has now become the main target of observatory opponents.

*To whom correspondence should be addressed.

Development of the observatory has been subject to intense scrutiny throughout, and plans have been modified in many ways to address public concerns. In the 6 years since authorization, an international facility has been established, which is at the cutting edge both in its telescope technology and in its sensitivity to the environment. Those responsible at the observatory and in federal agencies take pride in the amount of astronomical power located in the relatively small acreage assigned to the observatory. As a result, the project has an 'approval rating' of more than 65 per cent in Arizona (less than 20 per cent are opposed) and more than 90 per cent in Graham County. Yet the project has been the subject of endless debate and litigation. Why?

Opposition to the observatory

The opposition is now coming from activists within the Maricopa Audubon Society. They claim to be opposed to AICA but delaying tactics began well before AICA, and indeed were a motivation for the act. The current lawsuits suggest that opponents have little concern for the red squirrel, but are simply against telescopes, no matter how environmentally benign they are.

Given the overwhelming local support for the observatory, as well as its minimal environmental impact, how have opponents managed to maintain the controversy for so long? Sadly, the history of the project is littered with reminders of the problems facing science and public policy in the United States. Naive public acceptance of pseudoscientific (mis)information, the vulnerability of the legal system to incessant lawsuits, and the willingness of the media to transmit (perhaps preferentially) the more outrageous claims, have been exploited by observatory opponents to keep the pot boiling.

The methods of the activists include vigorous dissemination of misleading information (especially to funding sources), disruption of public functions (for example banners at commencement exercises alleging dire crimes against nature or humanity) and litigation, no matter how poorly based, to cause delay and uncertainty (for example the suit alleging violation by the Forest Service of the Historic Preservation Act, a case dismissed quickly by the district and appeals courts). The issue is always presented cleverly enough to fool some of the people at least some of the time. The red squirrel issue illustrates clearly the growing misuse of environmental or cultural laws, in this case the Endangered Species Act, to achieve unrelated special-interest goals.

Red squirrels

The anti-observatory campaign gained most of its impetus with the listing of the Mount Graham red squirrel as an en-

dangered species. The red squirrel is, of course, one of the most abundant mammals of North America. According to Hoffmeister¹, the Mount Graham red squirrel is a sub-population (he did not consider it unique enough to be a subspecies); it was left behind by the transition out of the last Ice Age.

The squirrel is threatened by inadequate food in times of cone crop failure. Its survival has been further jeopardized by large-scale logging and by successful colonization with introduced tree squirrels. The observatory is largely irrelevant to its future loss, as simple arithmetic on the areas of observatory (8.6 acres) and habitat (11,000 acres) demonstrates. The observatory footprint corresponds to a maximal reduction in red-squirrel carrying capacity of 0.8 squirrels (the earlier biological opinion conservatively estimated 2) when populations are at their highest (roughly 500) and to less than 0.2 squirrels when populations are low. The threat posed by the observatory has been and is being greatly exaggerated, despite clear evidence to the contrary.

Opponents also claim to be concerned about the effect of the observatory on red-squirrel behaviour, even though red squirrels usually thrive in the presence of human beings. Why the Mount Graham variety should be any different has never been explained — only repeated. More than 25 biologist-years of careful monitoring has, indeed, confirmed that there is no discernible effect on squirrel behaviour even in the immediate vicinity of construction. (In fact the "official" squirrel population has nearly doubled since construction began.) Again, the evidence has been largely ignored, perhaps just to create an interesting story where none exists. (Imagine the headline "Squirrels unaffected by telescopes".)

To use an endangered species to stop a project, it is necessary to identify such a species where the project is to be located — this is often referred to as the "snail-darter" strategy. In this case, the observatory must, of course, be placed at the highest elevation. From the outset, therefore, opponents claimed that the spruce/fir forest above 3,000 m was critical to the squirrel, even though, according to Hoffmeister¹ and the historical data given by Spicer *et al.*², the principal habitat (where most squirrels lived) was in the mixed conifer and transition zones well below the spruce/fir, and extending as low as 2,400 m. Since that time, biologists studying the squirrel have confirmed that the main population lives outside the spruce/fir zone. However, as a result of all the misinformation and pressure from observatory opponents, we now have a squirrel 'refugium' in the spruce/fir where the squirrel is found mainly when it is abundant but from which it seems to be absent when the population is low.

Clearly, the environmental impact of the observatory has been exaggerated, and the measures to protect the squirrel distorted, to halt the project. Some have sought to justify the situation by using the squirrel as a surrogate for the entire ecosystem. But as can readily be seen from the figure, the mountain has already been so compromised as to render questionable the need for such a surrogate. Certainly, the areas above 2,700 m could never qualify as wilderness because of roads and other artefacts.

The experience of the Mount Graham observatory raises serious questions as to the appropriate application of the Endangered Species Act. It is one thing to use the act to protect a truly endangered species from a real threat. It is quite another to use it to stop projects that have no significant environmental impact. It is this kind of misuse that is fueling the campaign against the act.

Unfortunately such distinctions are lost on observatory opponents, who continue to exploit the act to cause delay and unnecessary expense to the project. The costs for lawyers, professional protesters, protection from threatened damage to telescopes and so on must now run to millions of dollars, paid ultimately by donations to environmental organizations and by taxpayers through the federal agencies and the University of Arizona. All this ostensibly to 'save' a few acres from desecration by scientific research. Yet how many real environmental problems remain untouched? The costs to the Endangered Species Act, up for reapproval next year, and to the credibility of environmental organizations, might be even higher.

The whole situation cries out for the development of rational and achievable conservation goals. It would, for example, be helpful if the endangered-species listings took into account biological priority, and if quantitative measures of significant impact were defined. The current confusion on these topics — and consequent misuse of the laws — is building an anti-environmental backlash, with greatest risk of damage where the biological priority is highest. If Mount Graham illuminates this problem and brings about a more rational approach, this may well transcend even its importance to astronomy. □

Bruce Walsh is in the Department of Ecology and Evolutionary Biology; Roger Angel and Peter Strittmatter are at the Steward Observatory, University of Arizona, Tucson, Arizona 85721, USA.

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Immune cells and their interactions

Many insights derived from the immune system apply elsewhere, as *Nature's* conference, "The immune system as a model organ" (Boston, 7–8 November) learned. But in other respects, the immune system remains unique.

Boston. Half-way through a talk on lymphocyte homing and adhesion, Tim Springer (Harvard Medical School) took two dark lumps from his pocket and threw them at the projection screen. After a resounding thud, they stuck for several minutes, before sliding slowly downwards. The resemblance to the live lymphocytes he had just shown on video was startling, even if the latter normally roll before they adhere.

The relationship between lymphocytes and 'splat-balls' (as they proved subsequently to be called) may be only skin-deep. But might the parallels between the cells of the immune system and those elsewhere in the body be more than superficial? Certainly, the ease with which such cells and the cancers that affect them can be cultured have led to their intensive study.

Of course, some molecules of the immune system, like the elaborate antigen-specific receptors found on B and T cells, have no exact equivalent elsewhere. But as Dan Littman (University of California, San Francisco (UCSF)), Arthur Weiss (UCSF) and John Cambier (National Jewish Center for Immunology and Respiratory Medicine) showed, they may nevertheless induce effects very like those of other receptors when they have bound a ligand (MHC plus peptide in the case of the T-cell receptor, antigen in the case of the B-cell receptor).

In both cases, ligands induce rapid activation of an Src-family tyrosine kinase, which phosphorylates several related motifs on the receptor, forming a binding site for another tyrosine kinase called Zap-70, which in turn is activated by phosphorylation. *In vivo*, some of these steps may precede ligand binding, but the result is the same: a series of intracellular signals activating phospholipase C γ , phosphatidylinositol-3-OH kinase, and the MAP kinase cascade.

More conventional receptors such as that for epidermal growth factor induce very similar events. But the T-cell receptor goes further: its effects depend on its affinity for its ligand. Too low an affinity, and the cell in question dies by apoptosis; but too high an affinity also leads to cell death or anergy. Only receptors of intermediate affinity induce positive selection. The basis for this distinction is not fully understood, but selected T-cells appear to recognize peptides that are receptor antagonists, and the thymic ligand density is also important (Michael Bevan, University of Washington).

Other systems mediate a wide range of responses in a different way. The ErbB group of receptors, for instance, are all closely

related to the epidermal growth factor receptor (Yosef Yarden, Weizmann Institute, Israel). But each member of the family can form a heterodimer with any of the others, and each heterodimer has a distinct affinity for several different ligands, allowing very complex behaviour.

In other cases, closely related receptor subunits bearing identical Jak-type kinases can nevertheless phosphorylate distinct transcription factors of the Stat family (George Yancopoulos, Regeneron Pharmaceuticals). The basis for this selectivity appears to reside in the intracellular domains of the receptor subunits, which bring only the correct Stat(s) to the Jaks' attention.

Inside the cell, too, the parallels between lymphocytes and other cells are close. Numerous stimuli activate the immune transcription factor NF- κ B by inducing the degradation of its inhibitor I- κ B (David Baltimore, Massachusetts Institute of Technology (MIT)). But who would have thought that a very similar system would control dorso-ventral axis formation in *Drosophila*? For all its generality, however, the system is far from fully understood. As yet we can only guess why mice lacking the p50 subunit of NF- κ B are in most respects perfectly healthy, while those lacking other subunits perish at an early age.

Sometimes, generality can be exploited. The immunosuppressants cyclosporin A and FK506 interfere with T-cell activation by blocking the action of a calcium-dependent phosphatase called calcineurin. But both drugs must bind to small proteins called cyclophilins to act. Can a dimer of immunosuppressants bring together two proteins that do not normally interact, if the proteins are tagged with cyclophilins? It can, and the resulting research tool promises to be very useful (Gerald Crabtree, Stanford University Medical Institute).

Although the immune system was once thought to be unusual, in that its cells interacted in solution, it is increasingly clear that this is only partly true. Even formation of a blood clot begins with a site-specific interaction between fibronectin and the integrin receptors on platelets, triggering a network of intracellular signals that culminates in the activation of focal adhesion kinase (Joan Brugge, ARIAD Pharmaceuticals). And the rolling process studied by Springer is important precisely because it allows lymphocytes to form the interactions responsible for adhesion.

Such contacts are even more important in the bone marrow, where they direct the

earliest steps of immune cell differentiation. Even there, though, it is unlikely that they involve the exact three-dimensional specificity of the interactions specifying the initial formation of mesoderm in the vertebrate embryo (Douglas Melton, Harvard) or the patterning of different cell types in the nascent spinal chord (Thomas Jessell, Columbia). As in the immune system, both secreted and cell surface cytokines are important; the finding that brain-derived neurotrophic factor directs the innervation of the vestibular apparatus, but not the inner hair cells of the ear, while the opposite is true of neurotrophin-3, underlines the specialization of such trophic interactions (Rudolf Jaenisch, Whitehead Institute, MIT).

Whether cells are killed by virtue of their position or the affinity of their T-cell receptor, however, the pathway by which they die is similar. But instead of being a model, the immune system in this case follows lines first uncovered in the worm *Caenorhabditis elegans* (Robert H. Horvitz, MIT). In this organism, 1,090 cells are formed, of which 131 die by apoptosis, a form of cellular suicide triggered by the action of a protease encoded by the *ced-3* gene. In the remaining cells, the process is blocked by the *ced-9* gene, whose mammalian homologue is *bcl-2*, and overexpression of *bcl-2* indeed dramatically increases the number of circulating B-cells (Suzanne Cory, Walter & Eliza Hall Institute of Medical Research).

Even here, though, the immune system holds surprises. Overexpression of *bcl-2* does not preserve T cells from apoptosis (Cory). Moreover, while TNF α itself induces apoptosis in many cell types, an antibody to TNF α blocks the ability of lipopolysaccharide to rescue T-cells from the wave of death that normally limits their response to an antigen, suggesting that in this case TNF α may be anti-apoptotic (Philippa Marrack, National Jewish Center for Immunology and Respiratory Medicine).

So is the immune system a model organ? Clearly, the point can be pushed too far. It would be foolish to compare the ways in which lymphocytes and neurons store information, for example. And the parallels between AIDS (where persistent activation of non-infected cells increasingly seems to be important — Anthony Fauci, National Institutes of Health) and organotropic diseases such as hepatitis are fairly limited. But in other respects, so much of what has been learned about immune system has been or can be applied to other organs that the answer can only be yes.

Nicholas Short

Mucking up movements

Robert C. Malenka

It is generally thought that activity-dependent changes in synaptic strength mediate learning and memory, but experimental evidence for that view is limited. To establish such a link it would help to identify a synaptic or biochemical step that is unique to synaptic plasticity, but is otherwise silent — if disruption of the step selectively blocks both synaptic plasticity and learning and memory, then a strong case can be made that the two are intimately related.

The development of gene-targeting techniques offers the chance to try to do just that, and there has been a flurry of activity to 'knock out' mouse genes encoding proteins that may underlie synaptic plasticity and examine the effects on behaviour. On page 237 of this issue and in papers published last month in *Cell*, Conquet *et al.*¹ and Aiba *et al.*^{2,3} describe the consequences of deleting a subtype of metabotropic glutamate receptor, mGluR1. They show, for the first time, that the loss of a prominent form of synaptic plasticity in the cerebellum is accompanied by severe motor impairments. Moreover, both groups find the mice to be deficient in hippocampal-dependent learning tasks. An examination of hippocampal long-term potentiation (LTP) tends to confuse rather than clarify the role of mGluR1 in LTP, however, because the two groups obtain different results.

Glutamate receptors

Synaptic plasticity in the mammalian brain is routinely studied at excitatory synapses that use the neurotransmitter glutamate. Based on their pharmacological, electrophysiological and molecular properties, glutamate receptors can be classified into two classes, termed ionotropic and metabotropic glutamate receptors⁴. Ionotropic receptors are ligand-gated, cation-specific channels, and can be further subdivided into those that are and those that are not activated by *N*-methyl-D-aspartate (NMDA). Because of the availability of specific antagonists, much is known about the synaptic function of these receptors and their involvement in synaptic plasticity.

Metabotropic glutamate receptors, which were discovered only a decade ago, are coupled to various second messengers through G proteins. At least seven subtypes (mGluR1–mGluR7) have been identified by molecular cloning, and they are commonly divided into three subgroups according to molecular and biochemical criteria. Antagonists for mGluRs have become available only recently, although none of them is

subgroup- or subtype-specific. So less is known about the specific physiological functions of this receptor family, members of which are now known to occur ubiquitously in the mammalian brain.

Both Conquet *et al.*¹ and Aiba *et al.*^{2,3} examined synaptic plasticity in the cerebellum and in the hippocampus in mice lacking mGluR1, the activation of which increases phosphoinositide turnover. The cerebellum is important for the control and fine tuning of movements which may occur through a motor learning process involving synaptic plasticity⁵. Cerebellar Purkinje cells express high levels of mGluR1 messenger RNA and receive excitatory inputs from parallel fibres and climbing fibres. Repetitive and simultaneous activation of these two inputs results in a long-term depression (LTD) of synaptic strength at the parallel-fibre/Purkinje-cell synapse. Previous pharmacological experiments suggested that mGluRs are necessary for the generation of LTD (ref. 6), but — importantly — they have little if any part in generating basal synaptic responses. So deletion of the gene for mGluR1 potentially provided an ideal test of the hypothesis that cerebellar LTD is important for motor behaviour.

This hypothesis is now strongly supported by the demonstration^{1,3} that LTD is substantially reduced or absent in mice lacking mGluR1, and that these mice exhibit a profound motor coordination deficit or ataxia which is reminiscent of that seen following severe cerebellar damage. Moreover, the electrophysiological effects elicited by the mGluR agonist *trans*-ACPD are absent from Purkinje cells¹. The behavioural deficit is all the more remarkable in that no gross anatomical defects are evident in these mice, and all basic electrophysiological properties of Purkinje cells remain essentially unchanged. Although the profound motor deficit may involve a constellation of subtle abnormalities in many brain regions, it is not unreasonable to conclude that the correlative changes in LTD and motor behaviour are in fact causal, and that cerebellar LTD is a fundamental mediator of aspects of motor learning.

As well as testing motor ability, Aiba *et al.*³ examined the cerebellar-dependent learning task of eyeblink conditioning in their mGluR1-deficient mice. In this classical conditioning protocol, a tone precedes a mild electric shock which causes an eyeblink. After a number of trials, the animal blinks in response to the tone alone. The mGluR1-deficient mice exhibited a modest impairment of the conditioned eyeblink response, even though the unconditioned response to the shock

was normal. These results cannot distinguish between a cerebellar performance deficit and a specific impairment in cerebellar motor learning, but they are consistent with the idea that cerebellar LTD has some important, albeit not essential, part in this associative learning task.

Learning defects

Both groups also examined hippocampal-dependent learning tasks and hippocampal LTP which, like cerebellar LTD, may require mGluRs (ref. 7) (although the results supporting this hypothesis are controversial⁸). In two different learning tasks the mGluR1-deficient mice exhibited impairments which are unlikely to be due to their motor difficulties. Are these learning defects due to impairments in LTP mechanisms? Conquet *et al.*¹ report that the mutant mice exhibit normal LTP in CA1 pyramidal cells and in three other synapses which also exhibit NMDA receptor-dependent LTP. However, mossy fibre LTP, which does not require NMDA receptors, is markedly impaired. In contrast, Aiba *et al.*² find that LTP in CA1 cells is substantially reduced, although some preparations did exhibit normal LTP.

Notably, both groups find that *trans*-ACPD exerted all its normal electrophysiological effects in mice lacking mGluR1, including a presynaptic depressant effect⁴. This last result is intriguing — the impairments in mossy fibre and CA1 LTP must be attributed to the absence of a presynaptic mGluR1, because mossy fibre LTP is a presynaptic phenomenon⁹ and mGluR1s appear to be absent from CA1 cells². If mGluR1 is important for either form of LTP, it therefore exists as an additional presynaptic mGluR which either is electrophysiologically silent or cannot be activated by *trans*-ACPD.

The creation of mice lacking mGluR1 is another example of both the power and limitations of the gene knockout approach¹⁰. It permits questions about the relationship between proteins, synaptic function and behaviour to be addressed, but there are inevitably interpretational limitations due to possible developmental abnormalities and compensatory changes. The ideas that mGluR1s are required to generate cerebellar LTD, and that cerebellar LTD has a potential role in motor learning, have been strengthened considerably by the

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study of mGluR1-deficient mice. But the initial analysis of hippocampal synapses has not defined the enigmatic role of mGluRs in LTP more clearly, nor that of LTP in hippocampal-dependent learning and memory. As they stand, gene knock-outs are neither a panacea nor wasted effort for neuroscientists; rather they are useful adjuncts to the armamentarium of

techniques that will be required to understand synaptic plasticity and its involvement in behaviour. □

Robert C. Malenka is at the Center for Neurobiology and Psychiatry, Departments of Psychiatry and Physiology, University of California, San Francisco, California 94143, USA.

CHONDRULES

Small spheres of influence

Richard Ash

CHONDRULES are the most abundant macroscopic form of extraterrestrial material to fall to Earth, and may once have been the most abundant solid material in the young Solar System. Their origin, however, has remained a source of great controversy, and a conference last month* provided a forum for meteoritists, experimental petrologists and astrophysicists to discuss the formation and preservation of these objects, their relationship to other meteoritic materials and the nature of the material from which they were made.

Over 90 per cent of all meteorites falling to Earth are chondrites, which in turn consist of up to 85 per cent chondrules. Chondrules are spherical objects, up to 5 millimetres in diameter, whose mineralogy is dominated by olivine and pyroxene with textures and shapes that indicate that they were once at least partially molten. The relative abundances of refractory trace elements (such as scandium, yttrium and zirconium) in chondrules are approximately solar, telling us that chondrules have undergone no geological or cosmochemical processing, as this would separate these elements upon chemical grounds. The measured ages of chondrules are amongst the oldest recorded in any material, up to 10 million years older than the oldest igneous meteorites, evidence that they formed in the very earliest phases of Solar System history.

Since the first chondrule conference, held in Houston in 1982, much of the advance in the understanding of chondrule formation has been led by experimental petrologists, particularly Roger Hewins and colleagues (Rutgers Univ.) and Gary Lofgren (NASA Johnson Space Flight Center). Their recent simulations of the textural and chemical phenomena observed in natural chondrules now imply that the chondrule precursors were flash-heated to temperatures between 1,900 and 2,100 K. This temperature could only have been maintained for a matter of seconds, otherwise all poten-

tial mineralogical nucleation centres would have been destroyed and the resultant chondrule would invariably be glassy — a texture rare in natural chondrules. The hot droplets initially cooled rapidly, at 2,000 K per hour, but by the time they reached their solidus the cooling rates are estimated to have dropped to only 100 K per hour. Further evidence for rapid heat-

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Chondrules in the Sharps meteorite.

ing and cooling is the retention of relatively volatile elements, such as sodium, which in experiments can only be retained for a short duration in high-temperature regimes. To achieve such rapid heating and cooling each chondrule-forming event must have been on a localized scale.

A clear majority of conference participants favoured a nebula event for chondrule formation, envisaging 'dust-ball' precursors made up of well mixed presolar grains and Solar System condensates held together by electrostatic charges or an organic 'glue'. Later agglomerates may also have included recycled, broken chondrule fragments from previous chondrule-forming events which, in some cases, have been preserved as relict grains — olivines and pyroxenes out of chemical equilibrium with other minerals in the chondrule. Other evidence for multiple chondrule-forming events comes from paired and rimmed chondrules. The former are chon-

drules which have become joined whilst still molten. They appear as either adhering pairs or, more rarely, as chondrules completely enclosed within a larger chondrule host. Rimmed chondrules bear an outer rind of igneous material apparently formed by the sintering of dusty rims by a later flash-heating event. An extreme view was that there could have been tens or hundreds of generations of chondrule-forming events, destroying any vestige of the materials predicted to have been formed from the initial condensation of solids in the solar nebula (Conel Alexander, Carnegie Institution of Washington).

Only CAIs (calcium and aluminium-rich inclusions — refractory inclusions found in some carbonaceous and ordinary chondrites) are older Solar System materials than chondrules. The relative ages found using extinct-radionuclide techniques (^{26}Al – ^{26}Mg , ^{53}Mn – ^{53}Cr and ^{129}I – ^{129}Xe dating) all suggest that the chondrule-forming events started between 2 and 7 million years after CAI formation and continued sporadically over the next ten million years. Suggestions that the CAIs formed during energetic outbursts caused by material falling onto the young Sun's surface imply that the chondrule-forming event would have coincided with the t-Tauri phase of the Sun's evolution.

The heat source for chondrule melting remains uncertain. We know from the petrological data that we are looking for a very rapid heating source, but what? New detailed modelling of giant lightning flashes in the solar nebula, a previously popular candidate, showed these to be less viable than previously thought. Insufficient charge separation prevents a discharge powerful enough to melt the precursor solids.

Shock-wave heating is another possibility. The high dust densities required for the highly efficient chondrule-forming process point to its occurring in the dusty midplane of the solar nebula. The modelling of astronomical observations of young stars suggests that this region would be subject to shock waves leading to localized high temperatures and pressures. At the meeting, several candidates were proposed for the causes of the shocks, including highly energetic flares from the young, t-Tauri-like Sun. This is a particularly attractive proposition as it allows for multiple heating events over a ten-million-year period — the predicted lifetime for this stage of the Sun's evolution. Other proposed causes of shock were from the heterogeneous infall of dust clouds onto the Sun's accretion disk or from spiral density waves sweeping the nebula, leaving a trail of turbulence in their wake.

Although the consensus was that the chemistry of the chondrules requires small, chemically undifferentiated dust-ball precursors, there remain some who

*Chondrules and the Protoplanetary Disk, Institute of Meteoritics, University of New Mexico, Albuquerque, New Mexico, USA, 13–15 October 1994.

prefer an origin in a modified, geologically undifferentiated planetary body (Robert Hutchison, Natural History Museum, London; Ian Sanders, Trinity College, Dublin). The collision of completely molten planetesimals was advocated as a possibility, with the spray from the smashed body condensing and re-accreting onto the asteroid surface. The principal objection to this model, that complete melting of even a small body is extraordinarily difficult, still appears insurmountable. The presence of even a

small amount of solid material would inevitably lead to geochemical elemental fractionations.

Writing in this journal in 1877, H. C. Sorby originally described these particles as "drops of fiery rain". We have come a long way since then; but there is still much that remains unknown about these tiny, unique rocks. □

Richard Ash is in the Department of Geology, University of Manchester, Oxford Road, Manchester M13 9PL, UK.

SIGNAL TRANSDUCTION

How G proteins turn off

Roger S. Goody

THE importance of the heterotrimeric G proteins in transduction of signals that are received by cell-surface receptors has become abundantly clear in the past 20 years, and was recognized by the award of this year's Nobel Prize in Physiology or Medicine to Alfred Gilman and Martin Rodbell. Whereas earlier work concentrated on the physiological role of these proteins, and resulted in the establishment of the basic cycle of G-protein activity, more recently there has been significant progress in working out their detailed mechanism of action.

The most dramatic breakthrough has arisen from the determination of the structures of the α -subunits of two G proteins, $G_{i\alpha}$ (refs 1–3) and $G_{i\alpha 1}$ (ref. 4). The latest papers, by Sondek *et al.* on page 276 of this issue³ and Coleman *et al.* in *Science*⁴, provide spectacular evidence concerning the detailed chemistry of the G-protein GTPase mechanism. This mechanism forms the basis for the function of such proteins (GTP cleavage converts the protein from the active to the inactive form), so knowledge of it is central for understanding their mode of action. Furthermore, the new results and their interpretation are of direct relevance to the mechanisms of other closely related GTP-binding proteins (including the Ras superfamily and ribosomal elongation and initiation factors), and also those of mechanistically and structurally related ATPases and kinases, such as the energy-converting molecules myosin and F1-ATPase.

Apart from the determinations of the structures of G-protein α -subunits, in both the substrate- and product-bound states, the other principal starting point for the new studies was the discovery several years ago that the trimeric G-proteins, like several ATPases and kinases, can form stable complexes with GDP (or ADP) and aluminium in the presence of fluoride ions⁵. In the case of the G proteins, this results in formation of

a protein conformation that must be similar to the GTP-bound form, because they then behave as if they are normally activated. This and other considerations led to the suggestion that the negatively charged tetrafluoroaluminate ion occupies the position of the γ -phosphate group of GTP at the active site of the proteins⁶. Sondek *et al.*³ and Coleman *et al.*⁴ provide confirmation that this interpretation was correct, with the added bonus that a GDP-tetrafluoroaluminate-water complex is seen which resembles the structure of the putative pentacoordinate phosphorus intermediate, or perhaps transition state, in the GTPase mechanism.

The structures of $G_{i\alpha 1}$ and $G_{i\alpha}$ complexed with GDP and tetrafluoroaluminate show the aluminium in an octahedral complex. The equatorial plane of the octahedron is formed by a complex of aluminium with four fluorides. Two further ligands occupy the two axial positions. One of these is an oxygen of the β -phosphate of GDP, while density on the opposite side of the plane is identified in both papers as a water molecule. The geometry of this complex is similar to that of the pentacoordinate intermediate expected if, as was suggested earlier for the elongation factor EF-Tu (ref. 7) and p21^{ras} (ref. 8), the attack of water is in-line in an S_N2 reaction.

The complex is stabilized by a number of interactions with the protein. In terms of the GTPase mechanism, the most important of the interactions are ones which are newly formed in the GDP-tetrafluoroaluminate complex, or which may be stronger in this complex than in the GTP ground state. The two residues that appear to be vital are Gln 200 and Arg 174 in $G_{i\alpha}$ (Gln 204 and Arg 178 in $G_{i\alpha 1}$). The side chain of Gln 200 does not interact with the γ -phosphate group nor with the attacking water molecule in the ground (GTP) state, but in the GDP-tetrafluoroaluminate complex is seen to form stable hydrogen bonds to one of the

fluorides and to the apical water ligand, which is presumably in the position of the hydroxyl group derived from the attacking water molecule in the pentacoordinate intermediate in the GTPase mechanism. In the case of Arg 174 for $G_{i\alpha}$, an interaction with the β -phosphate group and the bridging β , γ -oxygen, present in the ground state, appears to be strengthened in the intermediate or transition-state analogue.

In $G_{i\alpha 1}$, the change in the corresponding residue (Arg 178) is more dramatic. It is not involved in direct interactions with the phosphate groups in the GTP γ S state, but interacts strongly with the fluorides in the tetrafluoroaluminate complex, and presumably with the γ -phosphate in the pentacoordinate or transition state, thus stabilizing it with respect to the ground state.

These results provide strong support for the principle that GTP hydrolysis is an in-line attack of water in an associative mechanism, and help us to understand how catalysis via stabilizing interactions with the intermediate and structurally related transition state occurs. But they do not show, at least directly, what happens to the proton of the attacking water molecule. In earlier work on p21^{ras}, it was suggested that the side chain of Gln 61 (corresponding to Gln 200 and Gln 204 in the two G proteins), together with the backbone carbonyl of Thr 35, activates the water molecule by hydrogen-bond formation; and that Gln 61, with the assistance of Glu 63, abstracts a proton⁹. Another suggestion concerning Gln 61 was that it stabilizes the transition state¹⁰. The most recent proposal for p21^{ras} is that the γ -phosphate group itself is the acceptor of the proton from the water molecule¹¹.

Sondek *et al.*³ now put forward a mechanism that incorporates elements of all these ideas. Water attacks the γ -phosphate in a concerted mechanism while a proton is being abstracted by the glutamine side chain, which in turn protonates an oxygen of the γ -phosphate, effectively shuttling the proton from the water to this oxygen. This leads to a two-pronged interaction of the 'wrong' tautomeric form of the glutamine side chain with the phosphate oxygens, which

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is perhaps an uncomfortable (but not entirely unprecedented) aspect. Alternatively, the authors consider a mechanism based on direct transfer of a proton from the water molecule to the γ -phosphate. In both models, the pentacovalent structure is stabilized by interactions with arginine and glutamine side chains, with the latter adopting different tautomeric forms in the two mechanisms.

Although these suggestions are consistent with much of the evidence available on the chemical mechanisms of GTPases, some facts and results do not fit into the picture. For example, the structurally related EF-Tu has an essential histidine at the position (204) corresponding to Gln 200. In p21^{ras}, replacement of Gln 61 by the unnatural amino-acid nitroglutamine,

in which the carboxamide is replaced by a nitro group, is reported to result in a protein with normal GTPase activity and even normal sensitivity to activation by GAP (GTPase activating protein)¹². This group is even less likely to act as a proton acceptor than glutamine and could not interact in an analogous manner with the pentacovalent intermediate, although some degree of stabilization may be possible. Perhaps the final clues to the mechanisms, which may not be identical for all GTPases, will come from a detailed study of the structure and kinetics of such modified proteins. □

Roger S. Goody is at the Max-Planck-Institut für Molekulare Physiologie, Postfach 10 26 64, 44026 Dortmund, Germany.

PALAEOCLIMATOLOGY

Damping seasonal variations

Paul Valdes

FROM a geological perspective, our present climate is unusually varied. For much of the distant past, climate appears to have been substantially warmer, particularly at mid- and high latitudes. For instance, during most of the past 250 million years, there have been no polar ice caps. The most recent period of warmth occurred in the early Eocene (about 50–55 million years ago), when geological data suggest that even in winter, continental interior temperatures remained above freezing. In this sense the Eocene climate is said to be equable, with no seasonal extremes. But studies using general circulation models (similar to those used for future climate-change predictions) simulate Eocene winter temperatures of -30°C or colder. This has led to a vigorous debate, with modellers questioning the reliability of the geological data and geologists querying the accuracy of the climate models. Lisa Sloan, writing in *Geology*¹, now suggests a possible solution to the problem: pay more attention to the details of the ancient landscape.

Evidence exists for large lakes in North America during the Eocene, and so Sloan included a representation of these lakes in a general circulation model simulation. The presence of the lakes acts to ameliorate the bitter cold in winter, producing a climate downwind of the lakes similar to that of maritime regions. The resulting warmth brings the models and data into much closer agreement. The changes caused by the lakes are as large as those caused by changing the concentration of atmospheric carbon dioxide, or changing ocean heat transport.

The work is the latest illustration of the (generally) beneficial interactions between climate modellers and geologists.

The story starts in the 1970s and early 1980s. Using a variety of palaeoclimate indicators, ranging from the habitats of ancient crocodiles to the types of plant fossil found in different regions, the geologists assembled a convincing picture of a climate that was considerably less extreme than the present². Indeed the Eocene was not the only equable period. Much of the Mesozoic era seems to have been very warm, culminating in a maximum in the mid-Cretaceous (about 100 million years ago). There was also evidence for high carbon dioxide concentrations during these periods³, thus adding to concern about future global warming.

These dramatic differences between present-day and past climate inspired a number of climate modellers to investigate the cause of the equability^{4,5}. Their results were surprising. Despite many attempts, no climate model was able to reproduce the necessary degree of equability, particularly in continental interiors far away from the moderating influence of the ocean. It was suggested that either there was a fundamental flaw in our understanding of climate and climate change, or the concept of an equable continental interior had to be abandoned⁵.

Piqued, the palaeoclimate community were spurred into further investigations. Using more sophisticated techniques based on the morphological characteristics of leaves, they confirmed that the climate of continental interiors was indeed equable⁶. The models appeared to be wrong. But there are some caveats. The most solid data are for the North American region. Less is known about the other continents. Further, even for North America, the data are heavily biased

towards the western side of the continent, so the problem of equable Eocene continental interior climates largely boils down to understanding the climate of the western United States.

This is where the influence of a large lake (in the vicinity of Green river in the western United States) becomes important. The addition of this lake into the climate model brings the simulations into closer agreement with the geological data. The lake has a large heat capacity, so it is slow to cool in winter and slow to warm in summer.

So is this the end of the story? No! Even with the lake, the seasonal range of temperatures is generally too large. Also the size of the imposed lake is potentially controversial. The general climate model has a resolution of 4.5° in latitude and 7.5° in longitude, and the lake occupies roughly two grid points. This is large — up to four times the size of one of today's Great Lakes — and will need clear geological validation. Further, it must be confirmed that all of the 'equable' data are of the same age as the lake. Should any of them be earlier or later than the ages of the lake, then the problem of equable climates will remain. New data are also beginning to arrive from Russia. This will be yet another test of the model predictions, in a region where the model indicates extreme winter cold.

The results also pose another question. When is a climate model wrong? The newer simulations are in better agreement with observation. It is therefore fair to say that the original climate model was wrong. However, the error was due not to inaccuracies in our understanding of the processes that control climate but to imprecise knowledge of the boundary conditions for the model — the amount and position of large expanses of water. The former error would spell doom to model predictions for future climate change; the latter just shows an incomplete understanding of climate history.

The agreement between model and data shows us that we should have some confidence in the ability of computer climate models. It also shows the power of combining modelling and data studies of palaeoclimates. We now have the beginnings of a self-consistent picture of the climate of the Eocene, where we understand both the climate change and its causes. □

Paul Valdes is in the Department of Meteorology, University of Reading, Whiteknights, Reading RG6 2AU, UK.

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Operons in eukaryotes follow the spliced leader

David Hirsh

MANY bacterial genes are organized into operons which are transcribed as polycistronic messenger RNAs. By contrast, eukaryotic genes were thought to be regulated individually and transcribed as monocistronic mRNAs. Last year, however, a group led by Tom Blumenthal announced the discovery that the nematode *Caenorhabditis elegans* uses both the prokaryotic and the eukaryotic patterns of gene organization and transcription¹.

Blumenthal and colleagues have now taken this work further (page 270 of this issue²). They describe how they have examined the *C. elegans* genomic database and found that at least a quarter of the genes seem to be organized into operons. Unlike prokaryotes, *C. elegans* processes its polycistronic mRNAs into monocistronic units before translating them, with *trans*-splicing playing an integral and specific role. Such phenomena have been seen in unicellular protozoan trypanosomes³, but it is astonishing to find them in a metazoan such as the worm.

The discovery of operons in *C. elegans* is one more in a series of surprising findings. The first related finding was that *C. elegans*, like trypanosomes, carries out *trans*-splicing⁴ (although, unlike trypanosomes it does not *trans*-splice all of its mRNAs and also employs *cis*-splicing). In worms, *trans*-splicing involves a 100-nucleotide-long spliced leader RNA. This RNA donates its 5'-terminal 22 nucleotides, the spliced leader, to a splice-acceptor site in pre-mRNA. This splice acceptor site is always upstream of the AUG translational initiation site. An upstream splice acceptor site lacking a donor site in the same RNA appears to be sufficient for *trans*-splicing to occur^{5,6}. The first spliced leader that was discovered, SL1, contains a conserved sequence found in all nematodes that have been examined. About 60 per cent of the genes studied in *C. elegans* encode mRNAs that are *trans*-spliced to SL1². Discovery of a second spliced leader (SL2) indicated that *trans*-splicing is gene-specific because only SL2, and not SL1, is *trans*-spliced to the 5'-ends of the mRNAs of the *gpd-3* and *tra-2* genes^{7,8}.

Hypotheses

In addition to SL2 *trans*-splicing, *gpd-3* and *tra-2* share a further common feature; each is about 100 base pairs downstream from another gene with the same 5'-3' orientation^{1,9}. On the basis of these two examples, Spieth *et al.*¹ proposed two hypotheses: that *C. elegans* genes are transcribed as polycistronic mRNAs, and

that SL2 *trans*-splicing occurs only on the downstream partner of closely spaced genes having the same orientation. Soon thereafter, a pair of genetically defined genes, *lin-15B* and *lin-15A*, were found to be in the same orientation and separated by only 107 base pairs^{10,11}. When their mRNAs were sequenced, the downstream *lin-15A* transcript was found to be *trans*-spliced to SL2. After mRNAs from two protein kinase genes, *kin-10* and *kin-13*, were discovered to be *trans*-spliced to SL2, genomic sequencing revealed another gene in the same orientation located about 100 base pairs upstream of each of these kinase genes¹.

Further support for the hypotheses of Blumenthal and colleagues came from a closer look at *gpd-3* and its neighbours. This gene is the 3'-terminal member of a cluster of three, the others being *mai-1* and *gpd-2*. All three have the same orientation, and each is separated by about 100 base pairs. *gpd-3* mRNA had already been shown to be spliced to SL2; *gpd-2* mRNAs were then found to be *trans*-spliced to both SL1 and SL2, which is consistent with *gpd-2* being downstream of *mai-1*; *mai-1*, the first gene in this cluster, is not *trans*-spliced. In summary, it emerged that six downstream genes near and in the same orientation as their upstream neighbours were all *trans*-spliced to SL2.

Artful use of the *C. elegans* genome sequencing project has now put the hypotheses on an even firmer footing. Zorio *et al.*² searched two megabases of genomic sequence in the *C. elegans* database^{12,13} for clusters containing genes about 100–400 base pairs apart from each other, in the same orientation and containing potential *trans*-splicing acceptor sites. Fourteen new clusters were found. The authors used reverse transcription and the polymerase chain reaction to see whether these genes were transcribed and to identify the *trans*-spliced transcripts. In all cases tested, transcripts of the downstream genes in a cluster turned out to be *trans*-spliced to SL2 or alternatively to SL2 and SL1. Those genes that were not downstream genes were *trans*-spliced to SL1 or not *trans*-spliced at all. In no case was the first gene of a cluster *trans*-spliced to SL2.

Evidence that a cluster is regulated as an operon, and that all genes in the cluster are transcribed as a polycistronic mRNA, came from transcriptional studies described in the paper published last year¹. It is particularly difficult to detect unprocessed mRNA precursors in nematodes,

perhaps because processing is fast and coupled to transcription. Although Spieth *et al.*¹ could not find the entire *mai-1/gpd-2/gpd-3* polycistronic precursor transcript, they isolated complementary DNAs encoding both *mai-1* and *gpd-2*, showing that these two genes at least are co-transcribed. It was probably possible to isolate the *mai-1/gpd-2* transcript because the *mai-1* polyadenylation signal differs from the nematode's polyA consensus sequence. This deviation might slow down 3' end cleavage and polyadenylation of the mRNA.

The same paper¹ also discussed DNA-transformation studies showing that transcription of the downstream genes depends on the upstream gene's promoter. Deletion of the *mai-1* gene and its upstream region eliminated transcription of *gpd-2* and *gpd-3*. Placing a heatshock promoter upstream of *gpd-2* restored transcription of both *gpd-2* and *gpd-3*. Mutating the promoter abolished transcription of *gpd-2* and *gpd-3*, showing that the one promoter was essential for transcription of both genes. It seems likely that all clusters using SL2 *trans*-splicing will be found to be transcribed as polycistronic mRNAs.

Coordinated regulation

The evidence thus far for operons in *C. elegans* fits the notion of coordinated regulation of the genes through synthesis of a polycistronic mRNA. One of the triumphs of the original operon hypothesis was that it explained coordinate expression of metabolically related genes, and several clusters identified in the *C. elegans* genomic database contain such genes. The *gpd-2* and *gpd-3* glyceraldehyde phosphate dehydrogenases and *mai-1*, which bears sequence similarity to the mammalian ATPase inhibitor, might share coordinate expression in response to ATP levels. The *kin-15* and *kin-16* genes encode similar transmembrane protein tyrosine kinases. New physiological relationships may be revealed when more genes located in operons are identified; and genes with related functions in development may be identified because they share an operon. In this regard, *lin-15A* and *lin-15B*, both of which are involved in intercellular signalling during vulval development, are noteworthy.

It seems that the position of a downstream gene and not its sequence *per se* confers SL2 *trans*-splicing specificity; for example, Spieth *et al.*¹ found that an SL1 accepting gene, *rol-6*, became SL2 *trans*-spliced when it was placed in the downstream position. *Trans*-splicing of downstream genes seems to depend on some aspect of polyadenylation of the mRNA of the upstream neighbour. Deletion of the polyadenylation signal at the 3' end of the *gpd-2* gene in the *mai-1/gpd-2/gpd-3* cluster led to accumulation of a *gpd-2/gpd-3* bicistronic transcript that was not *trans*-

spliced. This result suggests that *trans*-splicing does not precede 3'-end processing. Most likely, polyadenylation and *trans*-splicing will prove to be concerted reactions.

Cis-splicing of conventional introns seems to occur independently of either *trans*-splicing or 3'-end processing. In both the *mai-1/gpd-2* and *gpd-2/gpd-3* partially processed bicistronic transcripts, the internal introns had already been excised, even though cleavage and polyadenylation of the upstream gene and *trans*-splicing to the downstream gene had not occurred.

We are left with several questions, for instance whether clustering of genes into operons in *C. elegans* conserves space in the small genome. In turn, this raises the issue as to the direction in which evolution is proceeding. Is the *C. elegans* genome becoming more compact to achieve bacterial status, or is it expanding towards the higher eukaryotic monocistronic design with each gene preceded by its own set of transcriptional regulatory sequences? Perhaps *trans*-splicing and operons will also be found in those higher organisms with particularly compact genomes.

There is also the matter of whether the function of SL2 *trans*-splicing is limited to cleaving polycistronic mRNAs to monocistronic units. *Trans*-splicing of both SL1 and SL2 provides a defined 22-nucleotide sequence at the 5' ends of the processed transcripts. The current bet is that these spliced leaders also act as good translational initiation sites, although other functions are possible (nuclear export or protection of mRNA from degradation, for instance). From the database, it appears that about 30 per cent of the genes do not use *trans*-splicing. It will be interesting to see if their 5' ends already have good translational initiation regions, and how the sequences compare to those of spliced leaders. □

David Hirsh is in the Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons of Columbia University, 630 West 168th Street, New York, New York 10032, USA.

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How to help the corncrake

William J. Sutherland

THE rasping nocturnal call of the male corncrake, *Crex crex*, would have been familiar to most European country-dwellers in the nineteenth century. Now, however, the range of the species has diminished to such an extent that it is endangered. This elusive bird is still widespread in Europe, so it was appropriate that a workshop* held to discuss the

are present in the meadows. Corncrake populations have remained stable or declined more slowly where hay is harvested late because of the local climate or a high water table (N. Schaffer, Max Planck Institute, Radolfzell).

Another problem has been loss of tall-vegetation habitats because of changes in farming. Hay meadows have disappeared from many parts of western Europe as a result of new technologies encouraged by subsidies within the European Union for maize, poplars and sheep (J. Dixon, RSPB). Subsidies for cattle have encouraged more intensive field-management, allowing earlier cutting. The large populations of corncrakes in eastern Europe are expected to succumb to similar changes when the present economic chaos and hiatus in agricultural development come to an end.

Exclusion of grazing and mowing from strips of tall vegetation, for example at the fringes of wetlands, and changes in the timing and method of mechanized mowing, are the most promising conservation approaches (T. Stowe, RSPB). Delayed mowing reduces the risk of damage to both nests and chicks. Machine mowing from the periphery of a meadow towards its centre traps chicks and adults which are reluctant to cross open ground. Between 38 and 95 per cent of chicks are killed per mowing episode, and some chicks are exposed to several such episodes during their 35-day flightless period. Mowing from the centre of the field outwards reduces the mortality rate to 8–19 per cent as chicks are able to move away from the mower to adjacent unmowed areas. After the introduction of all these practices in 1991, the corncrake population of a nature reserve on the Isle of Coll, Scotland, has increased threefold. Schemes in Ireland and Scotland involving payments to farmers to modify mowing practices are also showing signs of success: the decline has slowed or been reversed in areas with the greatest uptake of grants.

The agricultural policies of the European Union are partly to blame for the corncrake's plight, but policy mechanisms also provide potential solutions. Within the framework of reducing excess production, there is the possibility of paying farmers to manage their land more sympathetically. In selected areas, such measures would not only help the corncrake, but also reduce nitrogen fertilizer inputs and agricultural surpluses, and benefit a range of other wildlife. □

William J. Sutherland is in the School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK.

IMAGE
UNAVAILABLE
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REASONS

Crex crex — elusive but endangered.

extent of the decline and how to reverse it had a pan-continental dimension.

Thirty one nations are home to breeding populations of corncrakes, and data on population trends are available for 26 of them. Declines have occurred in the past 5–10 years in 20 of the 26, and in six countries numbers of the bird have fallen by more than half (G. Rocamora, Ligue pour la Protection des Oiseaux). The worst story comes from Ireland — there, the number of corncrakes fell by 86 per cent between 1988 and 1994, and the species has disappeared from Northern Ireland.

Corncrake populations have been falling for about 100 years in parts of western Europe, coinciding with the introduction of mechanized hay mowing. Corncrakes nest on the ground and rear their chicks in tall vegetation, so mowing is highly destructive. All large populations of corncrakes (those with over 10,000 males) now occur in countries where hand mowing of hay remains a common practice (R. Green, Royal Society for the Protection of Birds). In some areas there has also been a trend towards earlier mowing, associated with a switch from hay- to silage-making and agricultural improvement of meadows. This increases the degree of overlap between the mowing season and the time at which many nests and chicks

*Birdlife International European Workshop on the Corncrake, Gdansk, Poland, 25–27 October 1994.

Proxy paradox for P-prediction

Kathleen C. Ruttenberg

PHOSPHORUS is a limiting nutrient in the ocean, and through its effects on plankton influences atmospheric chemistry, oceanic biogeochemistry and climate^{1,2}. Over short timescales, any number of factors can act singly or in concert to limit biological productivity in the ocean, but over geological timescales, as the sole significant source of phosphorus to the oceans is erosion of continental crustal material transported by rivers, P input ultimately limits the productivity. Filippelli and Delaney, writing in *Paleoceanography*³, have found evidence for substantial changes in P-burial in sediment cores retrieved from the equatorial Pacific Ocean, which they argue could reflect a net drop in riverine P-input to the global ocean during the Neogene period. A second provocative observation, from this and earlier work published last month⁴, is that the riverine P-input inferred from their records is at odds with the trend in continental weathering and river flux derived from the strontium isotope record.

In pursuit of understanding the ocean's role in controlling Earth's climate and global change, palaeoceanographers and palaeoclimatologists traditionally rely on chemical proxies of key elements which regulate ocean biogeochemical cycles. The Cenozoic increase in seawater ⁸⁷Sr/⁸⁶Sr is believed to reflect an increase in delivery of chemically weathered strontium to the ocean⁵, and Sr has been loosely used as a proxy for other weathering products delivered to the ocean, including phosphorus⁶. But Filippelli and Delaney have, for the first time, compared the Sr-derived continental weathering record with direct measurements of reactive phosphorus (reactive P), the latter ostensibly a direct measure of nutrient supply to the oceans. In a study of Neogene sediments collected during equatorial Pacific Legs 130 and 138 of the Ocean Drilling Program (Fig. 1), they find

significant, synchronous changes in P-accumulation rate across a suite of sediment cores retrieved from this high-productivity region. The most striking feature of the records is a drop in mean P-accumulation rate by about 60 per cent from a peak about 6 million years ago to a minimum about 2 million years ago (Fig. 2).

A similar trend had in fact been observed for total phosphorus in equatorial Pacific cores in an earlier study⁷, but the superior stratigraphic resolution and site-to-site correlation available for the cores studied by Filippelli and Delaney permit them to make a better case for an equatorial-wide trend. Also, they are careful to look at reactive-P burial, a better measure than total-P of the fraction of biologically cycled P, because its chemical form implies greater solubility and therefore bioavailability.

The high-productivity band that stretches across the equatorial Pacific represents only 3 per cent of total ocean area, but nonetheless is the locus of 18–56 per cent of new production in the oceans worldwide⁸. The sediments that underlie this zone consist mostly of the remains of organisms that lived in the surface waters. Filippelli and Delaney³ draw the first-order conclusion that the observed drop in phosphorus accumulation reflects a drop in productivity of overlying waters. As it is the supply of nutrients to the euphotic zone that limits the quantity of new production, they speculate further that this record could indicate a decrease in the overall supply of phosphorus to the ocean over this period. This chain of cause-and-effect reasoning is in the realm of the highly speculative, but Filippelli and Delaney do an admirable job of using the palaeoceanographic information at hand to assess first, if the signal observed in their cores is real; second, if the trend observed in the individual cores from the equatorial Pacific truly represents an

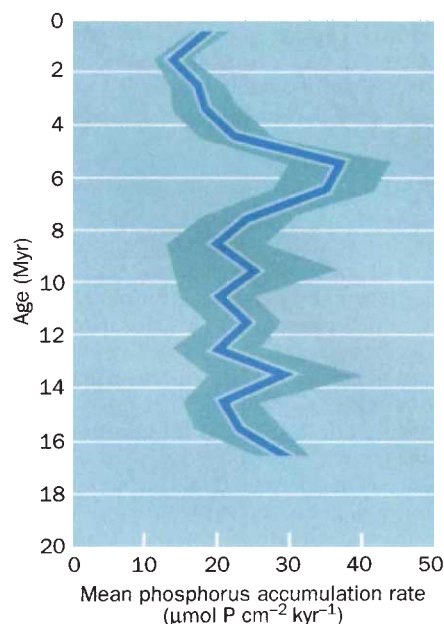


FIG. 2 Mean P-accumulation rates (line) and $\pm 2\sigma/\sqrt{n}$ standard deviations (shaded) for the equatorial Pacific cores shown in Fig. 1.

equatorial-wide phenomenon; and third, whether this regional trend might reflect a change in the global ocean phosphorus balance.

In making the case for an equatorial-wide phenomenon, Filippelli and Delaney discuss a range of site-specific processes which might explain the observed trends in any single core (for instance, shifts in prevailing currents overlying the sites, site migration through time changing the position of cores relative to the high-productivity equatorial belt, or water depth), some of which are more convincingly ruled out than others. The fact that the same trend of decreased phosphorus accumulation between 6 and 2 Myr ago is observed in six of the seven cores studied, from both the eastern and western equatorial Pacific, is indeed striking. Filippelli and Delaney make a plausible argument for an equatorial-wide event, and move on to assess its significance for the global ocean, using a mass balance approach.

A mass balance approach requires the assumption of steady state for the marine phosphorus cycle such that P-inputs are balanced by outputs, a reasonable assumption over the timescale of this study (millions of years). Within this framework, Filippelli and Delaney pose two questions. Is it possible to explain an equatorial-wide drop in P-burial rates by redistribution of P-burial elsewhere in the ocean? Or does this signal reflect a drop in riverine P-input rates to the ocean?

The first possibility is tested by assuming constant riverine input from 6 to 2 Myr ago, and contrasting the drop in P-burial rates for the equatorial Pacific with estimated P-burial rates in continental margins and high-latitude productive regions. Their point of departure is an assumption

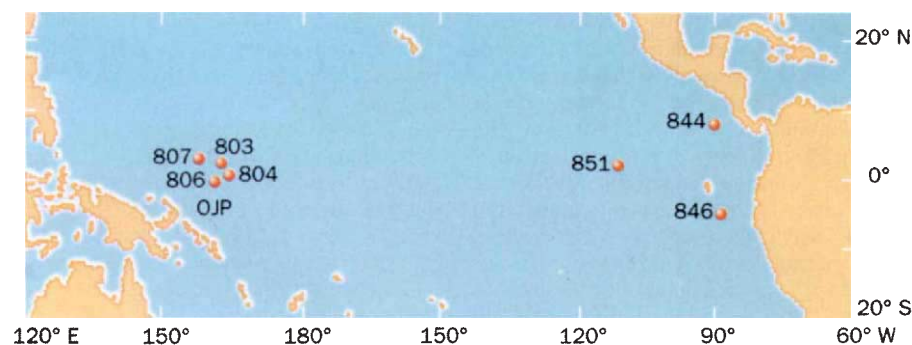


FIG. 1 Ocean Drilling Program station locations where P-accumulation rates have been determined: Leg 138 (eastern equatorial Pacific) sites 844, 846, 851; Leg 130 (western equatorial Pacific) sites 803, 804, 806, 807. OJP, Ontong – Java plateau.

that reactive-P burial is divided equally between continental shelves and the deep sea, and that burial is balanced by riverine input (steady state). Using a number of literature sources to constrain regional P-burial, Filippelli and Delaney conclude that redistribution alone cannot account for the equatorial Pacific trend. It must be said, however, that the river input flux and the regional burial fluxes are highly unconstrained estimates. For example, the P-burial flux in continental margin sediments has been estimated⁹ at up to seven times the value used in Filippelli and Delaney's model. If this higher flux estimate proves more realistic, it would reduce the global significance of the trend observed in the equatorial Pacific.

What about the alternative mechanism of variable riverine P-input rates? In this case, P-burial rates, used as a proxy for P-input rates, imply a substantial decrease in riverine P-input from 6 to 4 Myr ago. Strikingly, this trend is exactly the opposite of that implied by a popular proxy for the flux of weathered continental crustal material to the ocean: the $^{87}\text{Sr}/^{86}\text{Sr}$ record. (Strontium, isotopically homogeneous in the ocean because of its long residence time, is incorporated into marine carbonates without isotopic fractionation. The emerging consensus¹⁰ is that Cenozoic $^{87}\text{Sr}/^{86}\text{Sr}$ variation in sea water was driven by changes in the abundance and/or isotope composition of weathered continental material delivered to the ocean.)

The $^{87}\text{Sr}/^{86}\text{Sr}$ of Cenozoic sea water recorded in marine carbonates indicates that the riverine flux of dissolved weathering products to the ocean increased during most of the Cenozoic, and in particular from 6 to 4 Myr ago. Clearly, if the P-accumulation rate reflects riverine P-input rate, the $^{87}\text{Sr}/^{86}\text{Sr}$ record is at odds with the P-burial record in predicting riverine input of continental eroded material. How can these two conflicting records be reconciled?

Two explanations are advanced by Filippelli and Delaney: either the $^{87}\text{Sr}/^{86}\text{Sr}$ record reflects changes in source of weathered material, and not the rate at which it is weathered (an argument articulated previously by others^{11,12}), or phosphorus and strontium are not solubilized congruently during weathering reactions. Both are plausible explanations.

A third possibility is that, as strontium and phosphorus have very little in common chemically, their biogeochemical cycles should not be expected to mimic each other. These two elements lie at extreme ends of the spectrum with regard to reactivity in natural waters — witness their radically different ocean residence times, 3–4 million years for strontium^{13,14} but 20,000–80,000 years for phosphorus⁹. It is entirely possible that both are weathered congruently at the source, but the readily scavenged phosphorus gets held up in

transit to the ocean by biogeochemical reactions either in rivers or in the coastal zone, whereas strontium passes through unscathed. If so, the pelagic $^{87}\text{Sr}/^{86}\text{Sr}$ record could show a global signal of increased weathering input which would not be matched by phosphorus.

The paradox identified by Filippelli and Delaney raises the red flag for purveyors of proxies in unravelling biogeochemical changes recorded in deep-sea sediment records. On the other hand, although it may appear simpler to look at the 'real thing', there are pitfalls in interpretation here too. Interpreting the nature of the past ocean, and the input from continents to the ocean, by reading the record left in marine sediment cores is a tricky business. Extrapolation from a limited number of sites to larger regional or global areas is more the rule in oceanography than the exception. The systematic way in which Filippelli and Delaney lay out their argument bolsters the plausibility of their conclusions, and they raise intriguing questions which bear on our ability to construct realistic predictive models of bioactive elements.

Nonetheless, uncertainty in the evaluation of key fluxes makes it premature to draw conclusions about productivity per-

turbations and any effect this may have had on climate. This impasse serves to emphasize the importance of focusing efforts on better characterization of the geological, chemical and biological controls on fluxes of phosphorus into and out of the oceans. □

Kathleen C. Ruttenberg is in the Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Massachusetts 02543, USA.

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INTERSTELLAR MEDIUM

Dusting off the calculations

John Mathis

In spiral galaxies, interstellar grains absorb the visual and ultraviolet starlight and re-radiate its energy at far-infrared and millimetre wavelengths. The spectrum of far-infrared emission allows us to estimate the mass of interstellar matter, provided we know the absorption properties of the dust. But on page 243 of this issue, Agladze *et al.*¹ report laboratory measurements suggesting that the dust is far more opaque — and thus there may be a good deal less of it — than had been thought.

The chain of reasoning that leads from the far-infrared (FIR) data to a mass estimate for the associated interstellar medium goes as follows. First, we can estimate the average temperature of the emitting grains from the spectrum of the FIR; warm grains emit at short wavelengths. Next, the amount of FIR emission per kilogram of dust is known from the temperature, if we know the absorption properties of the dust. Kirchhoff's law gives $j_\nu = \kappa_\nu B_\nu(T)$, where j_ν is the dust emission per second per steradian per kilogram at frequency ν , κ_ν is the dust absorption cross-section per unit mass of the material, and $B_\nu(T)$ is the Planck function. Because the FIR escapes freely, unhindered by dust, the mass of grains

follows from the FIR luminosity of the galaxy or interstellar cloud and the emission per kilogram. Finally, the mass of gas is estimated by knowing the amount of dust per hydrogen atom in the solar neighbourhood.

Agladze *et al.*¹ have made laboratory measurements of κ_ν at millimetre wavelengths for a silicate that might be in interstellar grains. Some sort of amorphous silicate is known to be present in interstellar dust because of the strong 9.7- μm and 18- μm bands that are seen both in absorption and emission. Usually the elements silicon, iron and magnesium are depleted from the gaseous interstellar medium, presumably into the solid dust grains. There is so much cosmic oxygen that its abundance in solids, although somewhat uncertain, is more than enough to produce the silicates required for the absorption bands.

The process of estimating masses outlined above is actually more subtle than it sounds. For instance, masses of the interstellar medium in other galaxies have been drastically revised upwards within the last few years. In the mid-1980s dust masses were based on data from the 1983/84 Infra-Red Astronomical Satellite (IRAS), which had almost complete all-sky cover-

age with filters at 12, 25, 60 and 100 μm . However, the peak of the dust emission per hertz is at about 160 μm , so IRAS detects only the warmer dust. This is a serious defect, as the emissivity drops exponentially on the short-wavelength side of the maximum emission, making the measurements insensitive to much of the dust emission. More recently, observations at 600–1,300 μm , on the long-wavelength side of the emission, have become available. In this range the sensitivity to grain temperature becomes only linear rather than exponential. As a result, galaxies have been found to have about an order of magnitude more dust mass than was previously estimated, mostly cold and undetectable by IRAS.

Grain opacities have been thought to be well estimated by a model of dust² that reproduces the wavelength dependence of extinction well over the entire range of direct observations, 0.1–1 μm , and also the silicate features at 9.7 μm and 18 μm . In the model, silicates contribute about 40 per cent of the opacity at millimetre wavelengths, with carbon providing the rest. The model is based on astronomical and laboratory measurements and reasonable extrapolations from 100 μm to 1,000 μm on the basis that κ_{ν} is roughly proportional to ν^2 , which is suitable for many materials. It is this extrapolation that is challenged by Agladze *et al.*; they find a sixfold increase of the silicate opacity at 1 mm, with κ_{ν} proportional to $\nu^{1.3}$ in the millimetre range.

The increase in silicate opacity at far-infrared wavelengths has several implications. For a given grain temperature, the mass in the grains is inversely proportional to κ_{ν} . Another consequence is that when warmed by a particular radiation field, grains should be colder than formerly believed, as they can cool more efficiently by far-infrared emission. Hydrogen molecules encountering the cold grains are, in turn, cooled, and typical temperatures in molecular clouds will be lower than previously calculated. Clouds with gas barely above the temperature of the microwave background, too cold to emit even the lowest-energy radiation of CO, have been observed in absorption in the outer parts of our Galaxy³. The mass of molecular hydrogen may be five times that of the atomic hydrogen, so that it becomes dynamically important in the rotation of our Galaxy. Large millimetre opacities will help to explain how clouds can be so cool, although the density of cosmic rays, providing heating to the gas, also enters the picture.

The temperature sensitivity of the new opacity found by Agladze *et al.* is going to be an added complication in modelling the emission from dark clouds. But looking on the bright side, the decrease of the opacity as $\nu^{1.3}$ makes the spectrum of the millimetre radiation from circumstellar disks

surrounding very young stars much more understandable, as observations of most disks require a much more gentle increase than ν^2 (ref. 4).

It has been recognized that the far-infrared opacity might not be well understood, and this new measurement does not solve all problems. Opacity might be different for the diffuse interstellar medium than for the grains within cold, dark clouds in star-forming regions; even if the κ_{ν} of interstellar silicates were known, the geometry of the absorbing grain could alter the absorption per kilogram (imagine making solid spheres hollow and larger, with the same mass). Mixing of the carbon and silicate components within grains might change their properties. The Cosmic Background Explorer satellite, which last year obtained beautiful spectra of the Galactic far-infrared and millimetre emission while it determined the anisotropy of the cosmic microwave background, showed⁵ that the Galactic emission is well fitted by a mixture of grains with two temperatures and $\kappa_{\nu} \propto \nu^2$. The way with which the Galactic spectrum falls off for wavelengths above 200 μm limits the wavelength range over which κ_{ν} can vary as $\nu^{1.3}$.

But the best temperature measurements of dust masses in galaxies and circumstellar disks rotating around newly formed stars are in exactly the range of wavelengths covered by this laboratory measurement, and those masses might well be better estimated by using these new opacities than the more traditional ones — it might make them appreciably more reliable. In any case, more laboratory measurements of candidate grain materials would be most welcome. □

John Mathis is in the Department of Astronomy, University of Wisconsin, 750 North Charter Street, Madison, Wisconsin 53706, USA.

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Erratum

The caption to the figure on page 654 of the 20 October issue of *Nature* was missing in some editions. The figure accompanied the News and Views article "DNA repair: Incisions for excision" by Jan H. J. Hoeijmakers and Dirk Bootsma. The caption was as follows. "Tentative scheme for nucleotide-excision repair. The two strand incisions, shown by the scissors, are highlighted within the context of present knowledge (which stems from very varied work on mammalian and yeast mutants, cloning of genes, *in vitro* excision repair assays and from serendipitous findings)."

DAEDALUS

Carnot's demon

POLTERGEISTS, those disturbing spirit entities, have two main effects. They throw household objects about, and they make the room cold. Together, the two effects defy thermodynamics, which forbids spontaneous cooling below ambient temperature. Can a poltergeist be a Maxwell's demon? Can it arrange for an improbably large number of air molecules to hit an object on one side, and few to hit it on the other? This would punch the object across the room as required. The air around it, robbed of thermal energy, would cool down. Daedalus rejects this thermodynamic impossibility. Instead, he argues that the spiritual world occupies the same space as the material one, but is separated from it by some subtle barrier. A poltergeist is a local leakage between these two worlds. Its cold then has a simple explanation: the spirit world is much colder than the material one.

Thermodynamics can thus be rescued. A poltergeist has a temperature difference to exploit, and is a true heat engine. By comparing the work it does with the heat it absorbs, you could even deduce its thermal efficiency. Carnot's principle would then give you the actual temperature of the spirit world. Daedalus rather expects a value of ~ 3 K, the cosmic background temperature.

A Carnot poltergeist, however, could hardly abstract heat from matter by conduction. The molecules would have nothing to collide with. Radiation looks more promising. Information (such as telepathy, spirit-messages, and so on) is widely held to flow between the two worlds on occasion. And information can be identified with negative entropy, energy at a temperature, and nonequilibrium thermal radiation.

So Daedalus is considering practical tests. One suggestion is to measure the air temperature in a haunted house with recording thermographs. However, its radiation temperature seems more crucial. This should fall suddenly as haunting began. The air temperature would follow it down as a secondary effect. Radiation-sensitive thermocouples, or better, scanning thermal-imaging cameras with cryogenic calibration, should reveal the shape and temperature of the 'spiritual sink' as it opened to accept radiation.

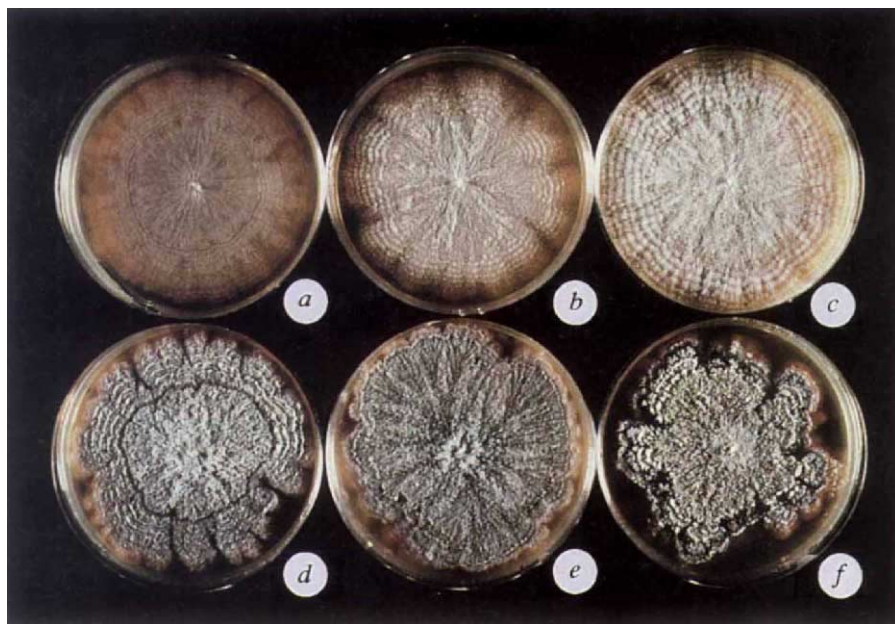
One camera should be trained on the human observers themselves. The apparent cooling of the room may be a sensory illusion: haunting may cool the human observer directly. The ghost may import heat into the spirit world through its one stable link to the material one: the human mind/brain combination itself.

David Jones

Missing link in tree disease

SIR — Dutch elm disease, the highly destructive fungal wilt disease of elms, spread by scolytid elm bark beetles¹, was unknown before 1900, yet two devastating pandemics have occurred this century, the first caused by the pathogen *Ophiostoma ulmi*, the second by the even more aggressive *O. novo-ulmi*². Each has spread

toms either on numerous native *Ulmus villosa* in the Balh and Lower Kullu valleys, or on scattered *U. wallichiana* in mixed forest at 2,000–3,000 m in the Upper Kullu valley. After extensive searches, however, I found small areas of active scolytid breeding in the bark of two *U. wallichiana* trees, from which I could



Colony types of the Dutch elm disease fungi on malt extract agar. a, *O. ulmi*, showing its typical smooth waxy colony type; b, c, *O. novo-ulmi*, showing its typical pale fibrous-striate colonies. d–f, The new Himalayan fungus, showing its distinctive fibrous dark pigmented colonies. The Himalayan fungus also differs from *O. ulmi* and *O. novo-ulmi* in its ability to produce synnemata (asexual fruiting structures) on this medium. Methods in ref. 5.

rapidly on a series of epidemic fronts, suggesting the involvement of an introduced or a newly evolved organism in each episode³. China has frequently been proposed as the source of the disease⁴, but a survey in 1987 revealed no evidence of it⁵. There has remained the possibility of a Himalayan origin³.

In a survey in Himachal Pradesh, western Himalayas, in September and October 1993, I found no elm-disease wilt symp-

tom obtain an apparently endemic Dutch elm disease *Ophiostoma*.

This Himalayan *Ophiostoma* possesses a unique combination of characters (see table) and a distinctive colony type (see figure). It also shares several important properties with *O. novo-ulmi*, the cause of the current pandemic, including the ability to produce very high levels of the protein wilt toxin cerato-ulmin (table). Inter-fertility tests show that it is strongly repro-

ductively isolated from both *O. ulmi* and *O. novo-ulmi* at the pre- and post-zygotic levels, although rare hybrids result from forced matings. It is evidently a new species of Dutch elm disease fungus³ which has probably evolved or become endemic in the Himalayas under conditions of geographical isolation.

An immediate question is whether this discovery will resolve the problem of the disease's origins. If the new Himalayan fungus is of only ancient affinity to *O. ulmi* and *O. novo-ulmi* and unconnected with this century's pandemics, then it and its potential hybrids may present an additional threat to current elm breeding programmes⁶ and other control projects. Alternatively, the recovery of occasional *O. novo-ulmi*-like colonies from forced *O. ulmi* × Himalayan *Ophiostoma* crosses (my unpublished data) indicates that *O. novo-ulmi* might have arisen via hybridization, consistent with my previous hypothesis³. A third possibility, that *O. novo-ulmi* has evolved directly from the Himalayan fungus, is suggested by the similarities between them. Genetic and molecular studies are required to distinguish between these possibilities, together with a full evaluation of the pathogenic ability of the Himalayan *Ophiostoma* and its potential hybrids.

Additionally, the discovery is an important opportunity to study the ecology and population dynamics of an endemic, as opposed to an epidemic, Dutch elm disease system. It may also offer new approaches to the control of the disease, as there may be natural constraints on the system in the Himalayas that could be exploited elsewhere for biocontrol purposes, such as parasites of the fungus or of its beetle vectors.

In a wider context, the discovery of a new and potentially very destructive pathogen species within a major forest system highlights the continuing risk posed to the world's forests by the importation of exotic pests and diseases. This is further exemplified by the effects of chestnut blight on the eastern hardwood forests of the United States⁷, of pine wood nematode in Japan⁸, and of *Phytophthora cinnamomi* on ancient forest communities in Australia⁹. Risks of such events are likely to be increasing with intensification of world trade, and are exacerbated by insufficient knowledge of indigenous diseases in many regions of timber export and a poor understanding of genetic variation in

COMPARATIVE PROPERTIES OF HIMALAYAN ISOLATES

Character	Himalayan <i>Ophiostoma</i>	<i>O. novo-ulmi</i>	<i>O. ulmi</i>
Radial growth rate (mm day ⁻¹), 20 °C	3.0–4.9 (25)	3.2–4.8	2.0–3.1
Growth temperature optimum (°C)	22–25 (6)	22–25	28–30
A : B mating-type ratio in nature	1:1 (25)	B type predominant	1 : 1
Perithecial (sexual stage) neck length (µm)	27–850 (16)	190–640	280–420
Cerato-ulmin toxin production index	600–1,700 (6)	490–600	0–4
Lesion formation in elm bark (mean lesion size; cm ²)	4.3–4.7 (6)	6.8–9.0	4.2–4.8
Pathogenicity (% defoliation at 12 wk)			
3 m <i>Ulmus procera</i>	30–55 (20)	30–80	10–20
4 m <i>U. x Commelin</i>	16–50 (18)	6–50	0–2

Number of Himalayan isolates tested are given in parentheses. Common ranges only shown. Methods in ref. 5. Known properties of *O. ulmi* and *O. novo-ulmi* are summarized in ref. 2.

Scientific Correspondence

Scientific Correspondence provides a forum in which readers may raise points of scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references.

most forest pathogens. To this may be added the unknown potential for evolutionary development in fungal pathogens, via hybridization, following sudden intermixing of related but previously geographically isolated pathogen species through human activities. All such risks are difficult to assess, especially when climate change¹⁰ and other environmental disturbances may be altering the balance of stress between pathogens and their hosts.

C. M. Brasier

Forest Research Station,
Alice Holt Lodge,
Farnham, Surrey GU10 4LH, UK

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Enigmatic statue

SIR — Ragge and Munier¹ propose the retrospective diagnosis of neurofibromatosis type-1 for a hellenistic statue. Linden, however, believes the statue was a votive offering². Actually, the case is more of an enigma than either of these views suggests.

The origin of this work of art is highly suspect, for it belonged to the Meyer-Steineg collection, the main part of which was gathered with an extraordinary rapidity by this collector-doctor during his only trip to Greece and Turkey in 1910. Meyer-Steineg asserted that the votive offerings in his collection came from Aesculapius' temple in Kos. The local people were grateful to him for his ophthalmological treatments (trachoma was then endemic on the island), and gave him many ancient artefacts³. Some of these were obvious fakes, others were altered to fit a medical amateur's tastes, and some may be authentic but are hard to date.

What happened to the collection during and after the Second World War is mysterious. It was said to have been taken to the Soviet Union, but it may never have left Iena, where it was found again in 1968 scattered in the garrets of a hospital^{4,5}. Anyway, it was a good occasion to let some of the questionable pieces vanish, among them the statue under discussion. This statue is known only through a photograph published, oddly enough, not in Meyer-Steineg's monograph on pathological figures (1912), but in a general history of medicine (1921), as Ragge and Munier rightly point out. According to the more recent catalogue, it is not known where

the statue was found, what it was made of, or what its dimensions were. Strictly speaking, it was probably not a statue but a small statuette⁶. Its authenticity can no longer be checked, nor can the overall appearance of the body and the details of the skin nodules be examined.

Assuming the statuette is ancient (in this case probably hellenistic), was it "an early three-dimensional teaching aid" (Ragge and Munier), a "votive offering" (Linden) or just a decorative artefact? Ragge and Munier rely on one of us to support a hypothesis that was actually discarded in the publication cited. All the objects supposed to be teaching aids are too small for such a use. But neither does the hypothesis of a votive offering rest on sound grounds. As they were prepared in advance for as many clients as possible, Greek votive offerings very seldom represent a pathological state. In such rare cases, they were generally personalized with the name of the donor. Pathological traits, on the other hand, are frequent on anonymous decorative figurines, especially on so-called 'grotesques'.

Statuettes with decorative functions sometimes led doctors astray in diagnosing ancient pathology. Similar nodules on fragments of statues have been misdiagnosed^{7–10}, as shown by the comparison with the complete statue of a Silenus in Museo Torlonia (Villa Albani, No. 704). The nodules are an artistic means of signifying the particularity of satyrs' skin. It is possible that Meyer-Steineg's statuette represents such a humanoid mythological being. Thus, for instance, a hellenistic statuette in the Louvre (D 1072), with a very similar torso, bears a tail which obviously does not represent a human disease.

Even if all these uncertainties were brushed aside, the retrospective diagnosis of neurofibromatosis-1 would still be only one of several possibilities — one strongly backed up by genetic considerations, but nonetheless weak in lack of literary and palaeopathological evidence concerning the presence of this disease in the ancient Greek world¹¹.

Danielle Gourevitch

Mirko D. Grmek

École Pratique des Hautes Études,
Ive Section (Sorbonne),
45 rue des Écoles, 75005 Paris, France

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Modern human origins

SIR — Waddle¹ has made an innovative attempt objectively to test two competing evolutionary models of modern human origins using fossil data. She suggests that there is stronger statistical support for a single-origin hypothesis than for the alternative hypothesis of multiregional evolution. Her analyses demonstrate that the observed taxonomic-distance matrix is significantly correlated with several of her hypothesis matrices, including those consistent with the single-origin and multiregional evolution models. But Waddle does not directly test the relative merit of these two models against one another in explaining her taxonomic distances.

We use the Dow-Cheverud² test to determine whether one of Waddle's hypothesis matrices has a significantly better fit with the observed taxonomic distances. To obtain *P*-values for the Mantel test of the matrix correlations, we use the randomization approach used by Waddle, but with the exact distribution from all possible 479,001,060 permutations of 12. The table shows the results, as well as a replication of two of Waddle's analyses. The two tests from ref. 1 listed in the table suggest that Waddle used two-tailed *P*-values derived by doubling the upper tail areas. This is questionable, both because the distributions under the null are generally asymmetric³ and because the alternative hypothesis is directional (that is we would not expect negative correlations between any of her distance matrices). Using only the upper tail areas, the randomization *P*-values of 0.0316 for regional continuity and 0.0046 for African replacement indicate that either hypothesis can adequately explain the observed distance data. The Dow-Cheverud test suggests that the hypothesis of a single African origin better fits the data. However, the randomization *P*-values are 0.2325 and 0.2263 for the lower and upper tails, respectively. As there is no directionality in this test (that is, there is no *a priori* reason to suspect either hypothesis will better fit the data) it is appropriate to use a two-tailed test. The combined *P*-value of 0.4588 shows that there is no statistical support for one hypothesis over the other.

The average taxonomic distance statistic used by Waddle makes some assumptions about the nature of trait variation and covariation that are open to question. Traits are assumed to be invariant within operational taxonomic units (OTUs); if this and other assumptions are met, then the expected squared average taxonomic distance⁴ will approach 2.0. However, Waddle's data apparently vary within OTUs, as she estimates parameters by averaging within OTUs. The expected

MATRIX CORRELATION TESTS FOR MODELS OF MODERN HUMAN ORIGINS

	<i>r</i>	<i>P_w</i>	<i>P</i> (lower)	<i>P</i> (upper)
Waddle's original distances				
Regional continuity	0.259	0.060	0.018856	0.031647
Single African origin	0.409	0.010	<0.000001	0.004551
Regional continuity versus single African origin	-0.109	-	0.232492	0.226333
Bias-corrected distances				
Regional continuity	0.358	-	0.002905	0.004898
Single African origin	0.275	-	0.011492	0.025424
Regional continuity versus single African origin	0.061	-	0.356905	0.360163

The column *r* gives the matrix correlations and *P_w* gives the associated probabilities from Waddle (if available). *P* (lower) and *P* (upper) give the lower and upper tail probabilities from the complete enumeration of 479,001,060 possible permutations. Matrix correlations for regional continuity versus single African origin are given as the correlation of taxonomic distance with the difference between design matrices (after standardization). Negative correlation indicates a better fit between taxonomic distance and the single African origin model, whereas positive correlation indicates a better fit with the regional continuity model.

value of $d^2(E\{d^2\})$ is then $(n_a + n_b)/(n_a n_b)$, where n_a and n_b are the sample sizes of the two OTUs. If sample sizes are small, because Waddle's distances (d_w) were not corrected for bias, two identical OTUs would still have a non-zero squared distance. If Waddle's average taxonomic distances are replaced with bias-corrected distances (d_c), equal to $(d_w^2 - E\{d^2\})^{1/2}$, or zero if $d_w^2 < E\{d^2\}$, and using Waddle's own form of inference (that is, choosing the model which gives the highest matrix correlation and the lowest *P*-value), we would be forced to pick the regional continuity model as the best supported (with $r = 0.358$ and $P = 0.0049$ versus $r = 0.275$ and $P = 0.0254$ for the single African origin model). The Dow-Cheverud test shows even less support for one model of modern human origins over the other ($P=0.7171$).

L. W. Konigsberg

A. Kramer

S. M. Donnelly

Department of Anthropology,
University of Tennessee,
Knoxville, Tennessee 37996, USA

J. H. Relethford

Department of Anthropology,
SUNY College at Oneonta,
Oneonta, New York 13820, USA

J. Blangero

Department of Genetics,
Southwest Foundation for Biomedical
Research,
San Antonio, Texas 78228, USA

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WADDLE REPLIES — Konigsberg *et al.* suggest that the one-tail permutational probabilities reported in my table are extreme for the two models of modern human origin. Although I agree with their conclusions on this point, and suggest that in future applications of this method, permutational probabilities be calculated several

times to ensure that the reported values are not extreme, probabilities are not particularly informative in interpreting the results of these matrix-correlation tests, because for almost all of the models tested in this and previous work, matrix correlations are significant. As I have discussed¹, there are large portions of the single origin and regional continuity design matrices which are either identical or very similar. These areas of similarity may contribute to positive correlations and statistical significance in matrix comparisons using each of the alternative hypotheses. The ideal method for testing competing hypotheses would be one that evaluates the strength of the different models solely on the basis of the differences between them (see ref. 1).

In theory, the Dow-Cheverud test used by Konigsberg *et al.* should accomplish this goal by allowing direct comparison of two competing hypotheses. As I have discussed¹, I did not use this method because my data are clearly spatially and temporally autocorrelated. Oden and Sokal^{2,3} have shown that this test results in spuriously high rejection rates when data are spatially and/or temporally autocorrelated. Konigsberg *et al.* use this method to show that neither of my basic single-origin or regional continuity models (using only one of my two sets of OTUs) provides a better explanation of my data on cranial variation. These authors have compared two of my models that have relatively low correlations relative to the other models tested, and did not address the single southwest Asian origin model, which achieved the highest correlations with the data. Therefore, their results do not change my conclusion that single-origin models provide the best explanation of cranial variation. At this time, I cannot comment on the portion of their paper discussing bias-correction for my average taxonomic distances, because the rationale behind the formula presented for the expected value of this statistic is unclear.

Finally, and most important, the

approach used by Konigsberg *et al.*, in which a single set of matrix correlations is used to evaluate a hypothesis, is fundamentally flawed. The conclusions in my Letter to *Nature*⁴ are based on a series of matrix correlation tests that all indicate stronger support for a single-origin model than for a continuity model. The use of such a series of tests is necessary given the amount of 'noise' present in my analysis. To be fair to both models, I used different formulations of OTUs, different formulations of geographical areas, and different weights applied to various parts of the models. The results of all my tests, demonstrating that this series consistently supported the single-origin model over regional continuity, convinced me that the single-origin model provides the better explanation of modern human origins.

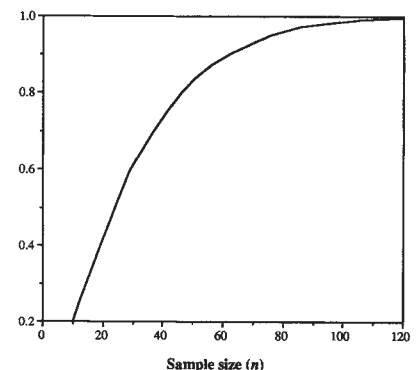
Diane Waddle

Duke University Medical Center,
Durham, North Carolina 27710, USA

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Rewards of promiscuity

SIR — Olsson *et al.*¹ described a remarkable relationship between mating behaviour of female lizards (*Lacerta agilis*) and viability of their offspring. The number of sexual partners of females was positively correlated with the hatching success of eggs, negatively correlated with the proportion of hatched young that exhibited malformations and positively correlated with survivorship of free-living juveniles. To test whether these differences in offspring viability were caused by nutrients in the ejaculates or offspring being sired by males with 'better genes', Olsson *et al.*¹ contrasted these results with those from females that mated repeatedly with the same male. The lack of significant correla-



Effect of sample size on power of a correlation analysis to reject the null hypothesis of the slope = 0 when the correlation is 0.40 ($\alpha = 0.05$).

tion between offspring viability and number of matings with the same male led the authors to conclude that genetic enhancement of offspring is probably the cause of increased viability of offspring of 'promiscuous' females. This conclusion, however, is open to discussion.

In the study of Olsson *et al.*, the number of females having mated repeatedly with the same male ($n=15$) was much lower than the number of females that mated with multiple males ($n=31$), resulting in a greater probability of detecting a significant correlation for the second group of females. Indeed, for two of the three variables measured (proportion of hatched young that exhibit malformation and survivorship of juveniles) the coefficient of correlation (r_s) was slightly greater in the first group (0.42 versus 0.33 and 0.41 versus 0.37, respectively). Further, examination of this dataset using a power analysis² demonstrates that the probability of detecting a significant correlation with only 15 individuals is low (0.3 assuming $r_s = 0.40$; see figure).

In conclusion, the available data are not sufficient to demonstrate that differences in the proportion of hatched young with deformities and survivorship of juveniles are caused by genetic enhancement of their offspring rather than increased nutrient acquisition by females mating multiply or some other factor influencing both the tendency of females to mate multiply and the viability of their offspring³.

Laurent Keller*
Institute of Zoology
and Animal Ecology,
Bâtiment de Biologie,
University of Lausanne,
1015 Lausanne, Switzerland

*Also at Zoologisches Institut, University of Bern, Wohlenstrasse 50a, CH-3032 Hinterkappelen, Switzerland.

OLSSON ET AL. REPLY — Is increased viability of offspring from our multiply mated female lizards due to a greater number of sexual partners (via sperm competition), as we suggested¹, or to a greater number of matings *per se* (via ejaculate nutrients or some other factor), as Keller suggests? Further analysis of the data supports our original interpretation.

Because the nutrient content of the ejaculate is likely to vary only negligibly among males, we can use our full dataset ($n = 31$) to compare offspring traits with number of ejaculates (matings) or number of partners. This procedure yields equal power in all tests. Number of matings was not significantly correlated with offspring mass ($r_s = -0.02$, $P = 0.90$), hatching success ($r_s = 0.09$, $P = 0.65$), or proportion of malformed offspring ($r_s = 0.02$, $P = 0.91$). Recapture rate correlates significantly with number of matings ($r_s = 0.40$, $P = 0.03$), but this correlation is not significant if the number of partners is factored out

in a Spearman's partial correlation analysis ($r_s = 0.33$, $P = 0.08$).

We can also control for differences in maternal investment between offspring, which could otherwise mask subtle effects of ejaculate nutrients. Because clutch size is correlated with hatching mass ($r_s = -0.48$, $P = 0.005$), we factored out this parameter in partial correlations. The number of matings still does not affect mean offspring mass ($r_s = 0.17$, $P = 0.36$), hatching success ($r_s = 0.04$, $P = 0.80$), or proportion of malformed young ($r_s = 0.04$, $P = 0.80$). In contrast, number of partners is significantly correlated with hatching success ($r_s = 0.58$, $P = 0.0009$), proportion of malformed offspring ($r_s = -0.38$, $P = 0.008$), mean offspring mass ($r_s = 0.46$, $P = 0.01$), and recapture rate ($r_s = 0.41$, $P = 0.03$). Thus, ejaculate nutrients or variation in maternal investment cannot explain our results.

Mats Olsson
Thomas Madsen
Richard Shine
School of Biological Sciences,
Zoology Building A08,
University of Sydney,
Sydney,
New South Wales 2006,
Australia

Annica Gullberg
Håkan Tegelström
Department of Genetics,
Uppsala University,
S-75007 Uppsala,
Sweden

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Chain mail

SIR — Goodenough and Dawkins¹ identify a quasi-religious mind virus (the St Jude chain letter) and postulate a class of postal parasites. By coating this mind virus with a purportedly therapeutic information package, they cleverly succeed in duplicating and circulating many copies. Such deceptive packaging typifies the dominant variety of postal parasites collectively termed 'junk mail' which shares with certain computer viruses and with popular song viruses the potential for completely clogging the systems in which they circulate.

A more complex mind virus is the idea of nuclear fission. It first infected O. Hahn and F. Strassmann in proviral form in December 1938. Hahn and Strassmann passed the provirus by letter to L. Meitner and O. Frisch, hosts in whom it assumed more typical morphology. Frisch passed it verbally to N. Bohr. Bohr crossed the Atlantic to the United States with L. Rosenfeld, infecting him along the way. The two vectors then divided. Rosen-

feld travelled to Princeton, where he infected among others E. Wigner, W. Lamb Jr and I. I. Rabi. Wigner infected L. Szilard. Rabi and Lamb hurried to Columbia and spread the infection to E. Fermi and generally among susceptible hosts (physicists). Bohr in the meantime went to Washington, Fermi followed, and together the two sufferers infected an entire conference of physicists, including E. Teller and G. Gamow. The infection then spread rapidly across the United States².

To acquire full virulence, scientific viruses must undergo independent replication. This doubting-Thomas test serves as an inoculant (note the withering effect of independent-replication failure on the cold-fusion virus). With experimental demonstrations in different institutions, publication of the Hahn–Strassmann report³ and confirming reports in this journal^{4,5}, the nuclear-fission infection spread rapidly throughout the scientific world.

Goodenough and Dawkins observe that 'chain letter' is a misnomer because such a letter forms, not a "linear array of links", but "an exponentially branching tree". But clearly "chain" refers to an exponential chain reaction. The nuclear-fission mind virus not only spread by this mechanism; its toxicity turned out also to depend on such a mechanism, a nice economy of form.

Unlike the relatively benign St Jude mind virus, the nuclear-fission information parasite did not merely induce mental and paper copies of itself but also took control of vast national machineries of production, first in the United States, then in the former Soviet Union (with K. Fuchs an important vector), then elsewhere. Nuclear weapons, a pathological end product of this parasitism, might be characterized as a form of gall, encapsulating the virus's toxic products. Perhaps paradoxically, after two appalling early episodes of gall lysis, these objects acquired such a fearsome (and deserved) reputation for toxicity that no one has dared lyse any since except under conditions of relative biological containment. Although governments have recently begun reducing their gall inventories, the nuclear-fission mind virus and its symbiont the nuclear-fusion mind virus continue to proliferate.

Richard Rhodes
609 Summer Hill Road,
Madison,
Connecticut 06443,
USA

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Signal transduction and regulation in smooth muscle

Andrew P. Somlyo & Avril V. Somlyo

Smooth muscle cells in the walls of many organs are vital for most bodily functions, and their abnormalities contribute to a range of diseases. Although based on a sliding-filament mechanism similar to that of striated muscles, contraction of smooth muscle is regulated by pharmacomechanical as well as by electromechanical coupling mechanisms. Recent studies have revealed previously unrecognized contractile regulatory processes, such as G-protein-coupled inhibition of myosin light-chain phosphatase, regulation of myosin light-chain kinase by other kinases, and the functional effects of smooth muscle myosin isoforms. Abnormalities of these regulatory mechanisms and isoform variations may contribute to diseases of smooth muscle, and the G-protein-coupled inhibition of protein phosphatase is also likely to be important in regulating non-muscle cell functions mediated by cytoplasmic myosin II.

THE expulsion of the newborn from the womb, the wheeze of asthma and the spasm of coronary arteries that weakens the heart are each the result of contraction of smooth muscle cells that form the main part of the walls of the uterus, blood vessels, airways, stomach, bladder and intestines. In spite of their important role in nearly all bodily functions, much of our knowledge of the mechanisms regulating smooth muscle is recent. This overview, intended for the general reader, emphasizes newly discovered mechanisms of regulation which may also function in non-muscle cells, as well as diseases of smooth muscle and the potential of their treatment by novel, molecular biological methods. The inevitable comparison of smooth with striated (skeletal and cardiac) muscles reveals, in addition to structural and functional similarities, the even greater differences between their respective signal transduction and regulatory mechanisms.

Activation of contraction and relaxation

The rise and fall in intracellular free Ca^{2+} are the principal mechanisms that initiate, respectively, contraction and relaxation in smooth, as in striated, muscles^{1,2}. From this junction, however, the two regulatory mechanisms diverge: in striated muscle, Ca^{2+} activates contraction by binding to the thin-filament-associated protein troponin³, whereas in smooth muscle Ca^{2+} binds to calmodulin, and the association of the regulatory, calcium-calmodulin complex with the catalytic subunit of myosin light-chain kinase (MLCK) activates this enzyme to phosphorylate serine at position 19 on the regulatory light chain (M_r 20,000; MLC_{20}) of myosin⁴. Phosphorylation of Ser 19 of MLC_{20} allows the myosin ATPase to be activated by actin and the muscle to contract^{4,5}. In addition to MLCK, calmodulin kinase II can also phosphorylate Ser 19 of MLC_{20} , but only at a very slow rate (1/20 of the $V_{\max}$ of MLCK) which is unlikely to contribute to the physiological initiation of contraction⁶. A fall in calcium concentration ($[\text{Ca}^{2+}]$) inactivates MLCK and permits dephosphorylation of MLC_{20} by myosin light-chain phosphatase, thus deactivating the actomyosin ATPase and causing relaxation⁷⁻⁹. One of the most important recent developments in this field has been the identification of secondary mechanisms of regulation that can modify, independently of $[\text{Ca}^{2+}]$, the activities of the phosphorylating and dephosphorylating enzymes. Inasmuch as vertebrate cytoplasmic myosin II is also regulated by phosphorylation/dephosphorylation¹⁰, these newly discovered mechanisms may also participate in important non-muscle cell functions such as cytokinesis and secretion.

Contractile regulation by thin-filament-associated proteins (for example, caldesmon and calponin), possibly through their phosphorylation by MAP kinase and/or other kinases, has been intensively studied¹¹⁻¹⁴, but remains to be conclusively proved. Furthermore, any directly Ca^{2+} -regulated system, even if present, could not be a rapid calcium switch, such as troponin in striated muscle⁴, given the long (about 300 ms) delay between the rise of cytoplasmic $[\text{Ca}^{2+}]$ and the onset of contraction in smooth muscle^{2,15,16,18}. Thin-filament-associated proteins would, at most, modulate the effect of myosin phosphorylation which alone is sufficient to initiate and maintain contraction^{4,17}.

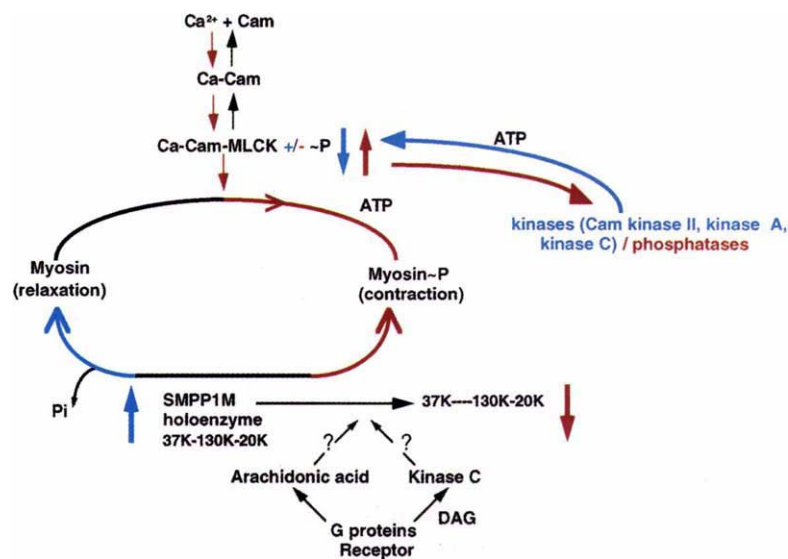
Excitation-contraction coupling

Electromechanical coupling operates through changes in surface membrane potential and their effects on cytoplasmic $[\text{Ca}^{2+}]$. The resting membrane potential of smooth muscle is, like that of other cells, negative (-40 to -70 mV, depending on cell type) with respect to the extracellular space; more positive potentials (depolarization) can open voltage-gated Ca^{2+} channels, causing Ca^{2+} influx to increase cytoplasmic $[\text{Ca}^{2+}]$ and trigger contraction^{2,18}. Tonic smooth muscles respond to excitatory stimuli with graded depolarization only, whereas phasic smooth muscles generate action potentials^{18,19} and some generate electrical slow waves as well²⁰. The depolarization-induced rise in $[\text{Ca}^{2+}]$ is due in part to the influx of extracellular Ca^{2+} through voltage-gated Ca^{2+} channels, but it is also likely that, as in cardiac muscle, the Ca^{2+} current carried by the action potentials releases additional Ca^{2+} through calcium-induced calcium release from the sarcoplasmic reticulum²¹. Indeed, the Ca^{2+} -buffering capacity of cytoplasmic Ca^{2+} -binding proteins exceeds

BOX 1 Ion channels in smooth muscle

Plasma membrane:
Voltage-gated
Directly ligand-gated (for example, by external ATP)
 Ca^{2+} -gated (K^+ , Cl^- channels)
ATP-gated (K^+ channels by intracellular ATP)
Modulators: arachidonic acid, other?
Sarcoplasmic reticulum:
InsP₃ receptor
Ryanodine receptor

FIG. 1 The electromechanical and pharmacomechanical pathways of excitation-contraction coupling. The pathways on the left-hand side (electromechanical and phosphatidylinositol-cascade-mediated), shown in red, operate by increasing cytoplasmic free Ca^{2+} ; this effect is reversed by removal of Ca^{2+} . The delays shown for the various steps of phosphatidylinositol-cascade-mediated Ca^{2+} -release are approximate, and were obtained at room temperature. The pathways (Ca^{2+} -sensitization and -desensitization) shown in blue on the right-hand side can operate at constant Ca^{2+} , and are the result of inhibition or activation of the enzymes phosphorylating (myosin light-chain kinase; MLCK) and dephosphorylating (smooth-muscle myosin light-chain phosphatase, SMPP1M) serine 19 on MLC_{20} . CICR, calcium-induced calcium release; MLC-P, phosphorylated myosin; MLCK-P, MLCK phosphorylated on site A; Ca-Cam, calcium-calmodulin; VGC Ca^{2+} , voltage-gated Ca^{2+} channel; SR, sarcoplasmic reticulum; InsP_3 , inositol 1,4,5-trisphosphate. Inhibition of voltage-gated calcium current by arachidonic acid (AA) and inhibition of InsP_3 production by hyperpolarization (E_m , membrane potential) represent bidirectional cross-talk between electromechanical and pharmacomechanical coupling. Short heavy arrows indicate increases or decreases in concentration or activity. DAG, diacylglycerol.

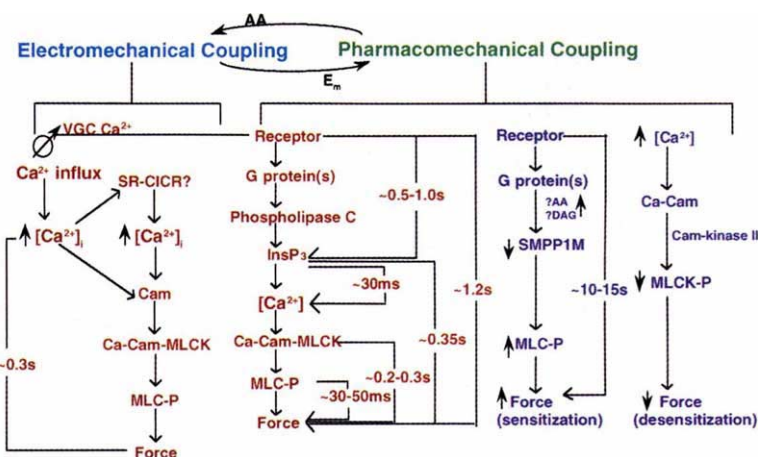


the amount of Ca^{2+} carried by an action potential²². A change to more negative membrane potentials, for example by drugs that open ATP-sensitive K^+ channels²³, can cause relaxation through inhibition of action potentials and reduction of the open-state probability of voltage-gated Ca^{2+} channels.

The plasma membranes of smooth muscles contain a great variety of ion channels, the distribution and properties of which vary among different (for example, intestinal, large or small vessel, uterine) tissues^{24,25}, contributing to the diversity of smooth muscles, but also precluding a comprehensive review here. Some of these ion channels can be regulated not only by the membrane potential (voltage-gated) or by the direct action of hormones and neurotransmitters (ligand-gated), but also by secondary messengers released by various agonists²⁵. Thus, Ca^{2+} released from the sarcoplasmic reticulum can activate Ca^{2+} -gated K^+ and Cl^- channels²⁶. The very different equilibrium potentials (E_K , E_{Cl}) of these ions in smooth muscles (E_K more negative,

E_{Cl} more positive than the membrane potential) can lead to the variable electrophysiological effects of agonists¹⁸, because the opening of Ca^{2+} -gated channels can result in hyperpolarization (K^+ channels), depolarization (Cl^- channels), or no change (opposite effects cancelling) in membrane potential. Similarly, arachidonic acid, released by excitatory agonists, can modulate K^+ -channel activity^{27,28} and inhibit Ca^{2+} influx through voltage-gated Ca^{2+} channels²⁷. The latter effect of arachidonic acid may account for the delayed inhibition of voltage-gated Ca^{2+} current by some agonists²⁹ that also release arachidonic acid. Ligand-gated channels are directly regulated by agonists, but are non-selective (can conduct several ions, mostly Na^+ , under physiological conditions); Ca^{2+} flux through these channels probably does not make a significant contribution to excitation-contraction coupling³⁰. However, the depolarization due to the opening of ligand-gated channels may cause significant Ca^{2+} influx by opening voltage-gated Ca^{2+} channels.

FIG. 2 Established and potential pathways modulating the Ca^{2+} -sensitivity of MLC_{20} phosphorylation and hence contraction. Ca^{2+} -sensitizing pathways are shown in red, desensitizing pathways in blue. Heavy arrows indicate increased or decreased enzyme (light-chain kinase or phosphatase) activity. Phosphorylation of MLC kinase (MLCK to MLCK-P) at site A by several protein kinases reduces its affinity for calcium-calmodulin (Ca-Cam) and, consequently, its activity at a given level of Ca^{2+} , whereas dephosphorylation of a phosphorylated MLC kinase would have the opposite effect. In contrast, inhibition of MLC phosphatase (SMPP1M) via a G-protein-coupled mechanism increases MLC_{20} phosphorylation and, consequently, force, at a given $[\text{Ca}^{2+}]$. The messengers mediating the effect of the plasma membrane-bound receptor-G-protein complex have not been definitely identified, but two of those implicated, arachidonic acid and protein kinase C, are shown. Mechanisms that would enhance dephosphorylation of myosin LC_{20} and relaxation through activation of MLC phosphatase have been proposed, but have yet to be experimentally demonstrated. Inhibition of SMPP1M is shown to result from reduced association between the 37K catalytic subunit and the targeting regulatory component (130K + 20K) of the enzyme^{74,75}. Abbreviations as in Fig. 1.



Pharmacomechanical coupling¹⁹, the second, physiologically highly important mechanism of excitation-contraction coupling in smooth muscle, operates through multiple cellular signalling mechanisms that can change the level of force without a necessary change in membrane potential (Fig. 1). The major mechanisms of pharmacomechanical coupling are Ca^{2+} release by inositol 1,4,5-trisphosphate (InsP_3) generated by the phosphatidylinositol cascade and modulation of the sensitivity to Ca^{2+} of MLC_{20} phosphorylation^{31,32}.

Sarcoplasmic reticulum

The sources of activator Ca^{2+} are both extracellular and intracellular. The sarcoplasmic reticulum (SR) is the physiological intracellular source and sink of activator Ca^{2+} in smooth³⁰, as in striated³, muscles. The Ca^{2+} pump of the SR is a sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase of M_r 100K with isoforms 2a and 2b; the 2a isoform is also found in cardiac and in slow striated muscles and the 2b isoform in the endoplasmic reticulum (ER) of non-muscle cells³³. The SR in smooth muscle also contains phospholamban, a low-molecular-weight phosphoprotein that regulates Ca^{2+} uptake by the SR in cardiac muscle³⁴. In several types of, largely phasic, smooth muscle (such as vas deferens, portal vein, taenia coli), the SR is scanty (2 to 3% of cell volume) and distributed mainly along the cell periphery. A relatively extensive central component of tubules and fenestrated sheets of SR and rough ER is prominent in smooth muscles which, in addition to their contractile functions, are actively synthesizing extracellular proteins (elastin, collagen, glycosaminoglycans; ref. 30). The lumen of the SR membrane is contiguous with tubules of rough ER and with the perinuclear space, and the central SR appears to form a continuous system connected with the peripheral SR. The peripheral SR can form 'surface couplings' with the plasma membrane: regions where the SR and plasma membranes come to within 8–10 nm of each other and are connected by electron-dense 'bridging structures'³⁰. But whereas in striated muscles the 'feet' connecting the terminal cisternae of the SR with the T tubules contain ryanodine receptor and dihydropyridine receptor proteins³⁵, the molecular identity of the bridging structures in smooth muscle is not known.

The Ca^{2+} -storage capacity of the SR is enhanced by the intraluminal Ca^{2+} -binding proteins calsequestrin and calreticulin. The former, the principal Ca^{2+} -binding protein in the SR of striated muscles, is present in the SR of some, but not all, smooth muscles^{36–38}, whereas calreticulin is a common component of the SR in smooth and of the ER in non-muscle cells³⁸.

InsP_3 and ryanodine receptors have been isolated from smooth muscle, localized to the SR and shown to act as ion channels^{35,37,39–41}. InsP_3 receptors are present in the central as well as in the peripheral SR⁴¹, suggesting that agonists can release Ca^{2+} from both sites known to store Ca^{2+} (refs 42, 43). The function of ryanodine receptors and the identity of the messenger(s) activating it in smooth muscle are not known, but the messenger is unlikely to be cyclic ADP ribose⁴¹. Experimentally, ryanodine receptors can be activated through calcium-induced calcium release²¹, but we still do not have proof of this mechanism operating under physiological conditions (for example, Ca^{2+} release by an action potential).

The role of InsP_3 as the messenger of pharmacomechanical Ca^{2+} release in smooth muscle has been firmly established through the use of specific inhibitors⁴⁴, measurements of agonist-induced InsP_3 production^{45,46}, and by kinetic studies showing that the rise in Ca^{2+} and contraction following photolytic release of InsP_3 from caged InsP_3 is consistent with the physiological role of this transmitter^{31,47}. Intracellular heparin blocks not only InsP_3 -induced Ca^{2+} release and contraction, but also the respective effects of excitatory agonists⁴⁴, confirming that InsP_3 mediates their effects. This important role of the phosphatidylinositol cascade in pharmacomechanical coupling contrasts with its negligible (or non-existent) role in excitation-contraction coupling

in striated muscle, in which Ca^{2+} release triggered by action potentials is not inhibited by heparin⁴⁸. Most excitatory agonists activate phospholipase C through a G protein (probably G_q): their effects can be mimicked by GTP- γ S and inhibited by GDP- β S (ref. 32). It is also likely that, as in other cells, receptor tyrosine kinases can also activate phospholipase C- γ present in smooth muscle⁴⁹ by phosphorylating it. The first major component of the commonly long delay (1–2 s) between agonist-receptor interaction and contraction is due to the time required for InsP_3 production, whereas the delay between InsP_3 release and the discharge of Ca^{2+} from the SR is relatively short⁴⁷ (Fig. 1). The second major component of the delay following Ca^{2+} release is due to prephosphorylation reactions, such as Ca^{2+} -calmodulin diffusion and/or activation of MLCK, and, to a lesser extent, the time course of myosin phosphorylation^{2,16,30–32,50}.

Cytoplasmic $[\text{Ca}^{2+}]$ can be reduced, causing relaxation, through several mechanisms. The rate and extent of Ca^{2+} pumping into the SR is sufficient to cause relaxation⁴², although other mechanisms of Ca^{2+} removal, such as the plasma membrane Ca^{2+} -ATPase⁵¹, and, to a lesser extent, the $\text{Na}^+/\text{Ca}^{2+}$ -exchanger^{52,53}, contribute to the maintenance of the steady-state levels of total cell calcium. As noted earlier, inhibition of Ca^{2+} influx, through hyperpolarization or by Ca^{2+} -entry blockers, can also reduce cytoplasmic $[\text{Ca}^{2+}]$ and cause relaxation.

An interesting form of bidirectional cross talk between electromechanical and pharmacomechanical coupling mechanisms has been recognized in smooth muscles. Not only can the second (and third) messengers (Ca^{2+} , arachidonic acid) of pharmacomechanical coupling affect ion channels and, consequently, the membrane potential and electromechanical coupling, but conversely, the membrane potential can also modulate the activation of phospholipase C by agonists: hyperpolarization inhibits it and thereby reduces the Ca^{2+} -releasing effect of agonists that are mediated by InsP_3 (refs 54, 55).

Regulation of Ca^{2+} -sensitivity

The contractile regulatory mechanisms of smooth muscle, myosin phosphorylation and dephosphorylation, lend themselves readily to secondary modulation of the Ca^{2+} -sensitivity of contraction (Fig. 2). Although such mechanisms had been proposed some time ago¹⁹, they were identified only recently. The use of Ca^{2+} indicators revealed that the force/ Ca^{2+} ratio is variable, and generally higher during activation by agonists than by a depolarization-induced increase in $[\text{Ca}^{2+}]$ (refs 56–58), indicating a ' Ca^{2+} -sensitizing' effect of agonists. Agonists (for example, α_1 -adrenergic and muscarinic, U44619, endothelin) can also increase force in permeabilized smooth muscles in which $[\text{Ca}^{2+}]$ is clamped with Ca^{2+} chelators and intracellular Ca^{2+} stores are removed, excluding the possibility of confounding effects due to intracellular Ca^{2+} compartmentalization and cytoplasmic Ca^{2+} gradients^{57,59,60}. Conversely, force can decline while cytoplasmic $[\text{Ca}^{2+}]$ is maintained^{61,62}, indicating a decrease in Ca^{2+} -sensitivity. Such increases and decreases in force at constant Ca^{2+} are now known to result from parallel changes in the activities of the phosphorylating and dephosphorylating enzymes and, consequently, in MLC_{20} phosphorylation^{63–65}.

Inhibition of the protein phosphatase that dephosphorylates Ser 19 of MLC_{20} is the main and perhaps only mechanism of G-protein-coupled Ca^{2+} -sensitization^{62–64}. Inhibition of the phosphatase increases MLC_{20} phosphorylation and hence the level of force at a given $[\text{Ca}^{2+}]$. The Ca^{2+} -sensitizing effect of agonists can be inhibited by GDP- β S and mimicked by GTP- γ S (refs 32, 59, 66), but the G protein(s) involved has yet to be identified. The fact that AlF_4^- can cause Ca^{2+} -sensitization in permeabilized smooth muscle⁶⁷ suggests the primary role of a trimeric G protein in Ca^{2+} -sensitization, because AlF_4^- does not interact with the monomeric Ras-family proteins⁶⁸. But the possibility that monomeric, Ras-family G proteins^{69,70} or

'cross-talking' tyrosine kinases⁷¹ contribute to Ca^{2+} -sensitization has not been excluded.

The agonists that can cause Ca^{2+} -sensitization can also activate the phosphatidylinositol cascade to increase both InsP_3 and diacylglycerol. This, in conjunction with the Ca^{2+} -sensitizing effects of phorbol esters (see below), raises the possibility that diacylglycerol is an early messenger of Ca^{2+} -sensitization and that the same G protein(s) is involved in the Ca^{2+} -releasing and Ca^{2+} -sensitizing pathways. But the efficiency of a given agonist in activating one or other of these pathways can differ greatly: for example, U44619 is a potent Ca^{2+} -sensitizer of rabbit pulmonary artery smooth muscle but has a very minor Ca^{2+} -releasing effect in the same tissue⁵⁷. Furthermore, high concentrations of verapamil can block the Ca^{2+} -releasing effect of phenylephrine in permeabilized smooth muscle without significantly inhibiting its Ca^{2+} -sensitizing action⁷². Thus, although the pathways are activated by identical receptors, the point where they diverge remains to be determined.

The agonist-G-protein complex that inhibits the dephosphorylation of myosin is physically separated from the phosphatase that is bound to myosin filaments^{73,75} and therefore a messenger or cascade must communicate the inhibitory signal to the inhibited enzyme. Both protein kinase C and arachidonic acid have been implicated as potential messengers. The main evidence for a role of protein kinase C is the Ca^{2+} -sensitizing effect of phorbol esters^{76,77}, which are well-known activators of kinase C, and the fact that the known Ca^{2+} -sensitizing agonists, as noted above, also activate phospholipase C and so release diacylglycerol (a 'natural' activator of kinase C) from phosphatidylinositol biphosphate and from phosphatidylcholine. The GTP- γ S-induced rise in diacylglycerol during Ca^{2+} -sensitization at constant $[\text{Ca}^{2+}]$ (ref. 78) is consistent with its role as a Ca^{2+} -sensitizing messenger. However, the inability of exogenous protein kinase C itself to cause Ca^{2+} -sensitization^{77,79,80} is not, unless this is due to the loss of a diffusible cofactor as the result of the extensive permeabilization required for diffusing the enzyme into cells. A protein phosphatase inhibitor that can be inhibited by protein kinase C could be such a 'lost' cofactor, as phorbol-ester-induced Ca^{2+} -sensitization is accompanied by an, albeit relatively modest, increase in phosphorylation of MLC_{20} at the myosin light-chain kinase site (Ser 19) (ref. 81). The possibility has not been excluded that phosphorylation of a thin-filament-associated protein (such as caldesmon), as the result of protein kinase C activation by diacylglycerol¹¹, contributes to Ca^{2+} -sensitization. But the effects of phorbol esters may be mediated through another mechanism such as activation of phospholipase A_2 and the release of arachidonic acid.

Arachidonic acid, released by agonists and implicated as a messenger in a variety of cell systems⁸², is a Ca^{2+} -sensitizing agent. It increases MLC_{20} phosphorylation and causes contraction of smooth muscle at constant Ca^{2+} by inhibiting myosin light-chain dephosphorylation⁶⁴. Ca^{2+} -sensitizing agonists and GTP- γ S increase free arachidonic acid in smooth muscle independently of $[\text{Ca}^{2+}]$ (ref. 78); the time course and magnitude of this increase appear to be consistent with, but do not prove, its role as a physiological messenger of G-protein-coupled protein phosphatase inhibition and Ca^{2+} -sensitization.

Phosphorylation of a specific serine residue in the region of the calmodulin-binding domain of myosin light-chain kinase (site A of MLCK) by several kinases reduces its affinity for calcium-calmodulin and consequently its phosphorylating activity⁶⁵. Phosphorylation of MLCK by one of these kinases, calmodulin kinase II, occurs *in vivo*, and is a physiological mechanism of desensitization, although it requires a higher cytoplasmic $[\text{Ca}^{2+}]$ than does activation of MLCK, owing to the lower affinity of calmodulin kinase II compared with that of MLCK for calcium-calmodulin⁶⁵. On the other hand, autophosphorylation of calmodulin kinase II could prolong its desensitizing activity beyond the duration of $[\text{Ca}^{2+}]$ elevation. Other desensitization phenom-

ena, such as the relaxation of permeabilized smooth muscle by cyclic GMP and by the cGMP-regulated kinase at lower (and constant) $[\text{Ca}^{2+}]$ (ref. 83) may be due to upregulation of myosin light-chain phosphatase.

Time is an important, but often neglected, variable affecting the relationship between myosin light-chain phosphorylation and the level of force. When developed very slowly, as in response to the Ca^{2+} -channel opener Bay-K8644, high force can be reached with very low MLC_{20} phosphorylation⁸⁴, as is also the case in response to phorbol esters⁸¹. Whether this represents an extreme case of 'latch' due to high levels of cooperativity, ADP accumulation, or some other mechanism, is not known, but it points to the importance of considering the rate as well as the level of force development in evaluating mechanisms of regulation. Unlike force, shortening velocity decreases with time and in parallel with MLC_{20} phosphorylation and energy consumption^{85,86}.

Phosphatase and kinase

The quaternary structure and the identity of the catalytic subunit of the protein phosphatase that is responsible for the physiological dephosphorylation of MLC_{20} has finally been determined^{74,75}. Several phosphatases can dephosphorylate isolated myosin light chains, but only those that dephosphorylate intact smooth-muscle myosin or its high-molecular-weight fragment, heavy meromyosin (HMM), can be involved in physiological regulation^{4,8,9,87}. The holoenzyme isolated recently from avian gizzard⁷⁴ and from mammalian pig bladder⁷⁵ smooth muscle is a trimer of two peptides of 130K and 20K and the 37K type-I protein phosphatase catalytic subunit. The avian holoenzyme is about two- to threefold more active than the catalytic subunit in dephosphorylating HMM in solution⁷⁴, and in permeabilized smooth muscle the relaxant effect of the mammalian holoenzyme is about two orders of magnitude greater than that of the catalytic subunit⁷⁵. The 130K subunit targets (or regulates) the catalytic subunit to myosin, and confers its high specificity for myosin. Its myosin-binding site has been localized to a 58K proteolytic fragment^{9,74}. Several non-muscle cells also contain the 130K 'targeting' subunit⁸⁸ and regulation of its association with the catalytic subunit could also mediate G-protein-coupled phosphatase inhibition of cytoplasmic myosin II.

Arachidonic acid dissociates the catalytic subunit from the holoenzyme while reducing its HMM phosphatase and increasing its phosphorylase-phosphatase activity⁶⁴. Such dissociation (or loosening) of the catalytic from the targeting (or regulatory) subunit, which is analogous to the mechanism originally proposed to regulate the glycogen-associated form of protein phosphatase-1, may account for the Ca^{2+} -sensitizing inhibition of myosin dephosphorylation by agonists and by GTP- γ S^{64,74,75,89}. The (inhibitory) phosphorylation of MLCK is also increased by GTP- γ S⁹⁰, and it is possible that this kinase is also regulated by the myosin-bound protein phosphatase.

The structure of MLCK is highly conserved among smooth muscles and probably among non-muscle tissues, but it is significantly different from that of the striated muscle enzyme⁴. An unusual property of the gene that encodes smooth muscle MLCK is that it also independently encodes telokin, a low-molecular-weight (18K) protein homologous to the C terminus of MLCK⁹¹, which is expressed in gizzard smooth muscle at a high abundance (80–90 μM), comparable to the myosin head concentration⁹².

Mechanism of contraction

The ultrastructural and biochemical bases of contraction are fundamentally similar in smooth and in vertebrate striated muscles. Cytoplasmic and plasma membrane-bound dense bodies, analogous to the Z-lines of striated muscle, contain α -actinin and are attachment sites for actin filaments³⁰. Smooth, like skeletal, muscle myosin is a hexamer consisting of two heavy and four light (two MLC_{20} and two LC_{17}) chains. Phosphoryla-

tion of the regulatory light chain (MLC₂₀) is the on-switch of actin-activated myosin ATPase activity. Substitution of a negatively charged amino acid for Ser 19 does not cause actin activation of myosin ATPase, suggesting a more specific, steric effect of introducing phosphate in this position⁵. Dephosphorylated MLC₂₀ inhibits and its removal increases basal myosin ATPase activity. However, normal motility requires the 26 C-terminal residues of MLC₂₀, indicating that it has a role apart from a simple on-switch in mechanical transduction⁹³. In differentiated smooth muscle, myosin is filamentous, regardless of whether it (MLC₂₀) is phosphorylated³⁰. The myosin filaments are somewhat longer in smooth than in striated muscle (2.2 rather than 1.5 μm ; ref. 30). In the absence of ATP, the crossbridges formed by the N-terminal (catalytic) head of myosin are attached to actin filaments, forming the characteristic rigor pattern¹⁵. The length-tension curve, force-velocity relationship⁹⁴, biochemistry^{4,95} and force transients^{15,50,96,97} of smooth muscle are consistent with a sliding-filament mechanism of contraction in which cycling crossbridges (myosin heads), energized by actin-activated ATPase, generate force and shortening. The relationship between the biochemical and the mechanical cycle is qualitatively similar to that in striated muscle⁹⁸: myosin crossbridges, bound to actin with high affinity in the absence of nucleotide, are detached upon binding ATP, hydrolyse ATP (largely in the detached state) and reattach into a low-force (weakly bound) state. This is followed by release of inorganic phosphate (P_i) and transition into force-generating (strongly bound) states terminated by ADP release, ATP binding, and repetition of the cycle. The rate-limiting steps (isomerization, P_i or ADP release) have not been definitively identified, but it is clear that ATP binding to the crossbridge and subsequent detachment are not rate-limiting^{15,95,96}. Cooperativity of the crossbridge cycle is manifested by the attachment of dephosphorylated crossbridges through the cooperative action of attached (rigor or phosphorylated) bridges¹⁵ and by product (ADP) release from a large number of crossbridges when only a fraction are phosphorylated⁹⁹.

Notwithstanding the considerable similarities of the molecular mechanisms of contraction in smooth and striated muscles, major differences exist between their macroscopic properties of energy metabolism, energy cost of force maintenance, and shortening velocity. The maximum shortening velocity and actomyosin ATPase activity of smooth muscle are slower by about an order of magnitude^{85,86,94,95} owing to differences between the respective myosins, as also shown in motility assays^{100,101}.

A striking aspect of the energy metabolism of smooth muscle is its ability to maintain high force at low energy (ATP) cost^{4,85,102}. This property resembles the catch state of some molluscan muscles in which force is also maintained at very low ATP cost, and muscles do not redevelop tension when released from their original length. A catch-like failure to redevelop force after a quick release also occurs in mammalian smooth muscle¹⁰³, and is also associated with low levels of MLC₂₀ phosphorylation ('latch'; ref. 102). The mechanism of this high-force, low-phosphorylation and low-energy-consumption state, renamed 'latch', is not known; in a two-state contractile model¹⁰⁴, it results from an increase in the ratio of crossbridge attachment/detachment constants. In view of the low ATPase activity and absence of force development following quick release during latch, such a change must be due to slowing of detachment. That slow detachment is due to dephosphorylation of MLC₂₀ on crossbridges in an AM (strongly bound actomyosin) state¹⁰² is unlikely, because a nucleotide-free AM (rigor-like) state must be short-lived in the presence of physiological ATP concentrations. However, dephosphorylation of attached crossbridges in a strongly bound, AM-ADP state⁹⁷, combined with cooperative reattachment of non-phosphorylated crossbridges through the cooperative action of attached crossbridges^{15,32}, could contribute to latch. The lifetime of the dephosphorylated AM-ADP state is expected to be prolonged

by the high affinity of smooth muscle myosin for ADP⁹⁷. Dephosphorylation, although it is necessary for the initiation of relaxation, is not its only rate-limiting step under all conditions, as it can be significantly faster than relaxation⁷. Any contribution to latch by the thin-filament-associated proteins caldesmon and calponin must be auxiliary, as smooth muscle can be fully relaxed by dephosphorylating MLC₂₀.

Diversity of smooth muscles

Unlike (striated) skeletal muscles, which are activated by acetylcholine released from one type of nerve, smooth-muscle cells can be stimulated or inhibited by many hormones and transmitters that activate an intricate complex of signal transduction mechanisms; the heterogeneity of the responses of different smooth muscles to this vast array of physiological and pharmacological agents has long been known. A given neurohormone can relax one, but contract another, smooth muscle, as, for example, α -adrenergic agonists relax intestinal and contract vascular smooth muscle through different, and now well understood, mechanisms¹⁰⁵. Smooth muscles in different vascular beds, in different size blood vessels, or even in different segments of the same blood vessel can vary greatly in their responses to excitatory and inhibitory stimuli. The fundamental mechanisms responsible for such diversity are beginning to be clarified through the identification and cloning of receptors, ion channels and other components of signal transduction. For example, the contractile response of many smooth muscles to several agonists (for example, α -adrenergic, muscarinic, angiotensin) is determined by the activation of seven-transmembrane-helix receptors associated with G protein(s) that activate phospholipase C, releasing InsP₃. Similarly, inhibition (relaxation) of smooth muscle can result from activation of receptors associated with G proteins coupled to adenylcyclase (G_s), generation of cAMP, and activation of the cAMP- or cGMP-dependent protein kinases⁸³.

Smooth muscles can be broadly classified into tonic and phasic types^{19,32} on the basis of intrinsic physiological properties determined by the coordinate expression of various ion channels and contractile protein isoforms. Phasic smooth muscles generate action potentials and have faster shortening velocities, attributable to the expression of fast myosin isoforms: a preponderance of the LC_{17a} isoform of the alkali myosin light chain and/or a seven-amino-acid insert in the myosin heavy chain¹⁰⁶. In contrast, tonic slow smooth muscles do not normally generate action potentials, have a relatively high content of LC_{17b} and have heavy chains which lack the seven-amino-acid insert. The slow myosin isoforms have a higher affinity for Mg-ADP which may contribute to the 'latch' state and slow shortening velocity^{97,106}.

Smooth muscle in disease

Both contractile and proliferative abnormalities of smooth muscle are major causes of disease, because mature smooth, unlike cardiac, muscle has the potential to replicate. Asthma and high blood pressure are manifestations of unwanted contraction of (bronchial and vascular) smooth muscle, due to an excess of excitatory agonists (histamine in allergy, for example) or enhanced responsiveness of smooth muscle to normal stimuli. The recently discovered multiplicity of contractile regulatory mechanisms suggests that their disorders, not only those of cellular calcium metabolism, may cause disease. Most drugs in current use (such as bronchodilators and vasodilators), however, are targeted to affect already well-characterized contractile regulatory mechanisms, such as the level of cytoplasmic Ca²⁺.

Proliferative disorders of vascular smooth muscle, some recognized as being due to alterations in proto-oncogene (*c-myc*, *c-myb*, for example)-mediated signal transduction, play a major part in coronary artery disease and are the subject of some of the most exciting developments in this field. One interesting recent finding is the frequent presence of high amounts of wild-type p53 protein in restenosed (narrowed after dilatation with a balloon

catheter) human coronary artery segments and its correlation with the presence of the human cytomegalovirus and its protein IE84. IE84 abolishes the ability of p53 to activate a reporter gene in human smooth-muscle cell culture, and it was suggested that this protein binds to and so blocks the normal inhibitory effect of p53 on cell-cycle progression, leading to excessive proliferation and vascular narrowing¹⁰⁷.

New therapies employing antisense oligonucleotides¹⁰⁸ or site-specific transfection, based on better understanding of the mechanisms regulating the cell cycle and of the importance of smooth-muscle cell proliferation in post-angioplasty restenosis,

are now being developed in animal models. Together with the discovery of previously unrecognized contractile regulatory mechanisms, the increased understanding of the fundamental mechanisms of smooth muscle function promises improved methods for the treatment of diseases of smooth muscle which are major causes of death and determinants of public health. □

Andrew P. Somlyo and Avril V. Somlyo are in the Department of Molecular Physiology and Biological Physics, University of Virginia Health Sciences Center, Jordan Hall 449, Charlottesville, Virginia 22908, USA.

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Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1

François Conquet^{*}, Zafar I. Bashir^{††}, Ceri H. Davies^{††}, Hervé Daniel[§],
 Francesco Ferraguti^{||}, Fabio Bordi^{||}, Karin Franz-Bacon^{*‡}, Angelo Reggiani^{||},
 Valerie Matarese^{||}, Françoise Condé[§], Graham L. Collingridge^{††}
 & Francis Crépel[§]

^{*} Glaxo Institute for Molecular Biology, 1228 Plan-Les-Ouates/Geneva, Switzerland

^{||} Department of Pharmacology, Glaxo Research Laboratories, 37100 Verona, Italy

[§] Département de Neurobiologie, CNRS and Université Paris-Sud, Orsay Cedex 91405, France

[†] Department of Pharmacology, The University of Birmingham, Birmingham B15 2TT, UK

Metabotropic glutamate receptor 1 (mGluR1) is a member of a large family of G-protein-coupled glutamate receptors, the physiological functions of which are largely unknown. Mice deficient in mGluR1 have severe motor coordination and spatial learning deficits. They have no gross anatomical or basic electrophysiological abnormalities in either the cerebellum or hippocampus, but they show impaired cerebellar long-term depression and hippocampal mossy fibre long-term potentiation. mGluR1-deficient mice should therefore be valuable models for studying synaptic plasticity.

Most excitatory synapses in the central nervous system (CNS) use glutamate as a neurotransmitter. Two main classes of receptors for glutamate have been characterized, the ionotropic and the metabotropic glutamate receptors (mGluRs)¹. The former group belongs to the superfamily of ligand-gated ion channels, whereas the latter group consists of receptors coupled, through G-proteins, to second messenger cascades. The physiological roles of the mGluRs are still largely unknown, although recent studies suggest a potential involvement of these receptors in synaptic plasticity². In particular, there is now some pharmacological evidence that mGluRs are involved in the induction of long-term potentiation (LTP)³⁻⁷, depotentiation⁸ and long-term depression (LTD)⁹ of synaptic transmission in the hippocampus, as well as in the induction of LTD at the parallel fibre (PF)-Purkinje cell synapses in the cerebellum¹⁰⁻¹³. But so far subtype-specific mGluR antagonists have not been identified to enable the function of any of the mGluR subtypes to be determined. As a complementary strategy for elucidating the physiological roles of specific mGluR subtypes, we have generated an mGluR1 gene-lacking mouse.

Generation of mGluR1-deficient mice

Homologous recombination-mediated gene targeting¹⁴ was achieved in HM1 (ref. 15) embryonic stem (ES) cells by inserting a *lacZ*/*neo*^r expression unit into the mGluR1 gene locus so that *lacZ* was in frame with the coding sequence, at the level of the second intracellular loop of the seven-transmembrane-helix domain¹⁶ (Fig. 1). Bearing in mind that the seven-transmembrane-helix domain and the intracellular part of the protein are essential to the function of the receptor¹⁷, it is unlikely that mGluR1 remains active when the gene is thus disrupted. The insertion of *lacZ* enables β -galactosidase (β -gal) expression to reflect endogenous mGluR1 gene expression. Two separate ES subclones, identified as having been targeted, were used to generate chimaeric mice. Four males transmitted the mutation when backcrossed with C57Bl/6 females.

[‡] Present addresses: Department of Anatomy, University of Bristol, Bristol BS8 1TD, UK (Z.I.B., G.L.C.); Department of Pharmacology, The University of Edinburgh, Edinburgh EH8 9JZ, UK (C.H.D.); DNAX Research Institute, Palo Alto, CA 94304, USA (K.F.-B.).

Behaviour of mGluR1 mutant mice

Mice homozygous for the mutant mGluR1 gene had a motor coordination deficit with impaired balance which could generally be referred to as ataxia. They also showed a wide-based standing position. Action tremor and errors in the metric of movements were constantly observed in the mGluR1^{-/-} but not in the mGluR1^{+/-} mutant mice. This behaviour became evident between the second and the third week after birth, with no distinguishing phenotype being apparent before. A complete neurological assessment of adult animals was made by Irwin screening¹⁸, which revealed a complete loss of the righting reflex but well maintained muscle strength. Spontaneous activity in a new environment was also investigated in mutant mice (Fig. 2). Homozygous mGluR1 mutant mice showed a significant reduction in total distance travelled, although this was not associated with a similar decrease in total horizontal activity (Fig. 2a, b). This indicates a maintained exploratory interest which is dramatically limited by the motor defect.

Learning capacity of mutant and wild-type mice was also investigated using the Morris test¹⁹. In the visible-platform task, mGluR1^{-/-} mice initially took longer than the wild-type group to reach the platform, but after three sessions they were as fast as the controls in locating the platform (Fig. 2c). We conclude that the homozygous mutant mice were able to associate the visible cue with the escape platform. In contrast, in the hidden-platform task, in which no cues are evident to indicate the location of the platform so that spatial ability is required for the learning task, mutant mice were never able to locate the platform (Fig. 2d). Because in the visible-platform task controls and mutants performed similarly after three trials, it can be concluded that the deficit in the spatial task was not caused by their motor impairment.

Anatomical studies

mGluR1 transcripts are widely distributed in the adult rat brain and are specifically localized to neurons²⁰. Most intensely labelled neurons are Purkinje cells of the cerebellar cortex and neurons in the hippocampus, olfactory bulb and thalamus. Histochemical analysis of mGluR1-mutant mice did not reveal any gross anatomical abnormalities in the hippocampus (Fig. 3a, b), cerebellum (Fig. 3f, j) or elsewhere (data not shown).

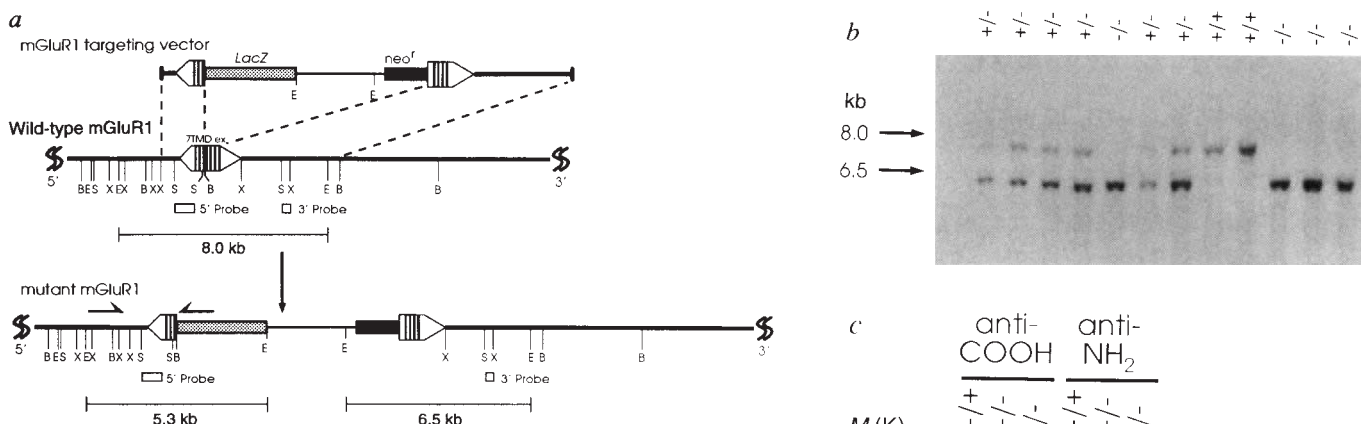


FIG. 1 Targeted disruption of the mGluR1 gene. **a**, Schematic representation of mGluR1 gene targeting. The location of introns and exons is incomplete but the single exon that spans the 7-transmembrane-helix domain (7TMD) is shown as 7TMDex.³³ The transmembrane segments are represented by vertical lines within 7TMDex. Mouse mGluR1 genomic fragments were isolated from a 129/Sv DNA library screened with oligonucleotides from the rat cDNA sequence¹⁶. The targeting vector was constructed by inserting a *lacZ*/*neo*^r expression unit³⁴ into a *Bgl*II site between the *Xba*I–*Bgl*II (1.1 kb) and *Bgl*II–*Bgl*II (6 kb) genomic so that *lacZ* was in frame with the mGluR1 gene coding sequence at the level of the second intracellular loop of the 7TMD (Ile 693)¹⁶. The two facing arrows represent positions of primers used for PCR analysis. B, *Bgl*II; E, *Eco*RI; S, *Sac*I; X, *Xba*I. **b**, Southern analysis of the mGluR1-mutated locus. Tail DNA from intercrossing of heterozygote littermates was collected according to standard procedure, digested with *Eco*RI and hybridized with mGluR1 5' (*Sac*I–*Sac*I) and 3' (*Sac*I–*Xba*I) internal probes. The wild-type allele gave rise to an 8.0-kb fragment and the mutant allele gave rise to a 5.3-kb fragment when hybridized with the 5' probe (not shown) and to a 6.5-kb fragment when hybridized with a 3' probe. **c**, Immunoblot analysis using antisera against the C-terminal (anti-COOH-mGluR1) and N-terminal (anti-NH₂-mGluR1) parts of the protein. Molecular weight markers are shown on the left. Positions of native mGluR1 and of the fusion protein are indicated by filled and open triangles respectively. Genotype is indicated above each lane: +/+, wild type; +/-, heterozygote; -/-, homozygous mutant.

METHODS. 2×10^7 ES cells were electroporated (Bio-Rad Gene Pulser) with 20 μ g of linearized targeting vector at 960 μ F and 250 V. G418 selection was applied (150μ g ml⁻¹) 24 h after transfection and resistant clones were isolated 10 days after selection. The homologous

recombination event was first detected in ES cells by PCR (data not shown) with a *lacZ* oligonucleotide (5'-AGTCACGACGTTGTAACACG-3') and a mGluR1 genomic oligonucleotide (5'-TTTCCGCTAACTACATACTT-3') and confirmed by genomic Southern blot performed as described above. Injection of ES cells into blastocysts was performed according to standard procedure³⁵. 10 μ g of DNA from the tail of the offspring were digested with *Eco*RI, run on a 0.8% agarose gel, transferred to nitrocellulose and hybridized with radiolabelled probes according to standard protocols. Polyclonal antibody against an inclusion protein containing a part of the N-terminal portion of mGluR1 (from Met 82 to Val 520)¹⁶ was made by overexpression in *E. coli*. Affinity purification of the antibody specific for mGluR1 was performed on a Sepharose-4B column coupled with antigen. Crude membrane preparations from mice cerebella were separated on 5–15% gradient SDS–PAGE and transferred to nitrocellulose (Amersham). The anti-COOH mGluR1 was raised against the C-terminal part of mGluR1 α . The membrane was first incubated with anti-COOH mGluR1 α ³⁶ (0.5 μ g ml⁻¹) or anti-NH₂ mGluR1 (1 μ g ml⁻¹) antibodies and then with a horseradish peroxidase labelled secondary antibody (Amersham); reacted bands were visualized by enhanced chemiluminescence (Amersham).

In addition, mutant animals showed intense β -gal activity restricted to the cell body of Purkinje cells (Fig. 3o). Expression can also be seen in hippocampal pyramidal neurons (Fig. 3g, h), whereas no β -gal activity was detectable in wild-type mice (data not shown).

Electrophysiology of the cerebellum

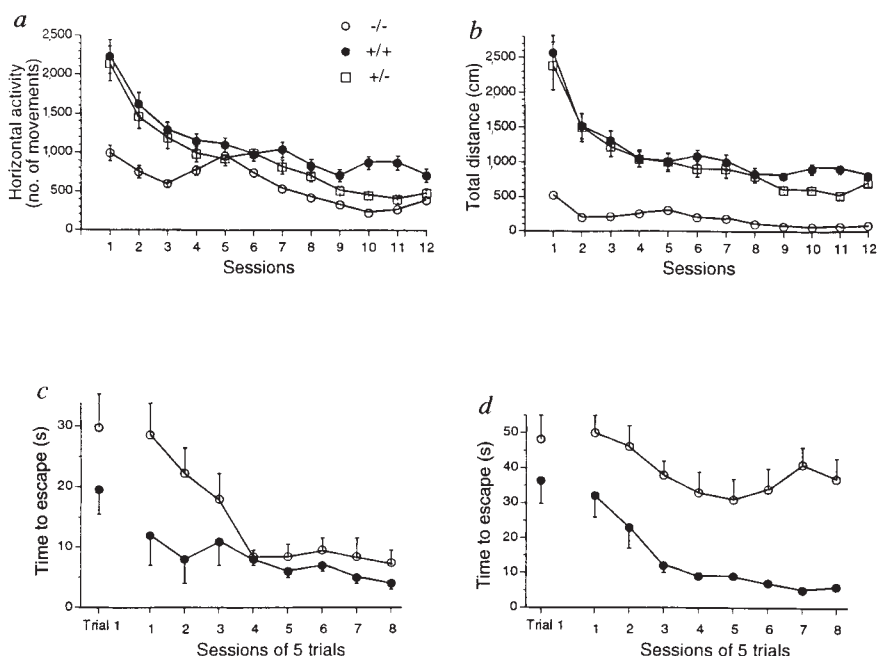
No differences could be detected between wild-type and heterozygous mutant mice with respect to active or passive membrane properties or synaptic responses. In homozygous mutant mice, the main subthreshold and suprathreshold voltage-dependent conductances of normal Purkinje cells, namely fast Na⁺ spikes, high-threshold Ca²⁺ spikes, the persistent and tetrodotoxin-sensitive Na⁺ current and the inward rectifier (Na⁺–K⁺) current, *I_h* (ref. 21) were, at least qualitatively, preserved. This was also the case for the kinetics, the linear current–voltage relationships and the pharmacological properties of PF-mediated excitatory postsynaptic currents (e.p.s.cs), of climbing fibre (CF)-mediated e.p.s.cs and of the inhibitory synaptic noise²². In particular, the all-or-none character of CF-mediated e.p.s.cs observed in all tested cells suggests that mutant Purkinje cells are normally mono-innervated by CFs, as described previously for adult Purkinje cells²³.

In wild-type Purkinje cells, recorded in the voltage-clamp mode and maintained at a holding potential of –70 mV, bath application of 100 μ M (1S,3R)-1-aminocyclopentane-1,3-dicar-

Immunoblot of total proteins and immunocytochemical analysis with a rabbit polyclonal antibody specific for the carboxy-terminal part of mGluR1 indicated that no protein could be detected in the hippocampus or cerebellum of homozygous mutant mice (Fig. 3d, l) when compared to wild-type mice (Fig. 3c, k). Furthermore, by using a polyclonal antibody raised against the amino-terminal part of mGluR1, two specific bands of proteins could be detected: the native and the mGluR1/ β -gal fusion protein. As shown by western blot analysis, only the fusion protein was found in homozygous mutant animals (Fig. 1c). Unlike in wild-type mice (Fig. 3m), immunocytochemistry with this antibody demonstrated the complete absence of mGluR1 immunoreactivity in the molecular layer of the cerebellar cortex of homozygous mutant animals (Fig. 3n). There was also an increased immunoreactivity in Purkinje cell bodies of mutant mice, presumably representing accumulation of the fusion protein which is no longer transported to the dendrites. In the hippocampus, the N-terminal antibody stained the entire hippocampus, with the exception of the cell body layers, and this staining was higher than in the surrounding tissue (Fig. 3e). In area CA3 there was staining associated with the mossy fibre pathway and a particularly pronounced staining of the more distal dendritic regions. In mutant mice, staining was restricted to the somatic region of CA3 neurons, granule cells and some neurons in strata oriens and radiatum of CA1, again presumably representing accumulation of the fusion protein (Fig. 3f).

FIG. 2 Spontaneous behaviour in a new environment (a, b) of wild type (filled circles; $n=7$), heterozygous (open squares; $n=7$) and homozygous mutant mice (open circles; $n=4$) is represented as horizontal activity (a) and total distance travelled (b). c and d, Learning tests. Homozygous mutant mice ($n=11$) were impaired initially at locating the visible platform but by the end of the second day they were as fast as wild-type mice ($n=11$) to reach the platform (c). An ANOVA with repeated measures showed that overall, wild-type controls were better than the homozygous mutant mice ($F(1,87)=3.2$; $P<0.01$). Post-hoc analysis (Bonferroni/Dunn, $P<0.05$) showed that the control group had significantly lower escape latencies compared to the mutant mice in sessions 1 and 2, but were not different in sessions 3–8. In the hidden platform task, the mutant mice ($n=7$) never learned to locate the platform, and they did not appear to improve during training, compared to wild-type mice ($n=7$) (d). Statistical difference was found between mutant and wild-type animals ($F(1,55)=9.6$; $P<0.01$). Post-hoc comparison indicated significant difference between the two groups from session 3 onwards. Each point is the mean $\pm$ standard error. Mean and s.e.m. of the first trial is also indicated for each graph to show the starting point of the learning curve.

METHODS. Animal activity was monitored in a computer-assisted Digiscan Animal Activity Monitor (Omnitech Electronics) by using a total of 24 infrared beams to measure over 20 different indices of behaviour in real time as described³⁷. Activity was monitored for a total period of 60 min with 12 time samples of 5 min each. Spatial learning was assessed in the Morris water maze¹⁹, adapted for mice. The maze consisted of a circular pool, 80 cm diameter, filled with water rendered opaque by milk and maintained at $23 \pm 1^\circ\text{C}$. The wall of the pool, 40 cm height, was painted white. A platform, 10 cm in diameter and submerged by 0.5 to 1 cm, represented the escape from the water. All around the platform and submerged about 3 cm in the water, we also placed a 14 cm diameter ring made in plastic and covered with a white gauze-like material. This ring allowed the mutant mice to get on the platform more easily once they reached it. Providing this help to the animals, we tried to eliminate possible interferences in the task due to the motor impairments found in the mutant mGluR1 mice. The ability of the mutant mice to learn the visible platform task makes us believe that we were successful in doing so. Although impaired in their movements on the ground, the mutant mice were as fast and efficient as the



wild-type mice in water. In a pilot study, we tested speed and distance swum in a one-arm water maze and found no difference between the two groups (not shown). The day before testing, the animals were given 2 min each of free swimming in the pool to acclimatize them to the water. In the visible-platform version of the Morris test, the platform was made visible by a small black cone attached to its top. The mice were placed in each trial in a different point of the pool to swim to the randomly located visible platform. Animals were allowed 60 s to locate the platform. If after 60 s, mice that had not found the platform were placed on it and escape latency was scored as 61 s. Once the subject found the platform, it was allowed to remain there for 30 s. Animals were given 10 trials a day, in 2 sessions of 5 trials each, for 4 consecutive days. Ten days after the visible-platform task, mice were trained on the hidden-platform version of the Morris test¹⁹. In the hidden-platform task, the cone was removed from the top of the platform and only distal cues present in the room surrounding the pool were available to the animals to locate the platform. The platform in this test was left in a fixed location throughout the 4 days of the experiment. Animals performed 10 trials a day, divided into two sessions.

boxylate ((1S,3R)-ACPD), a selective mGluR agonist, always induced a marked depression of PF-mediated e.p.s.cs (mean decrease was $49.48 \pm 3.62\%$) and an inward current of several hundred picoamperes in amplitude ($n=5$). These two effects were fully reversible after wash-out of the compound and, moreover, during wash-out, the inward current was followed by a transient outward current (Fig. 4A). Therefore, the effects of 1S,3R-ACPD in wild-type mice are fully consistent with those described in rats²⁴. In contrast, no effect of 1S,3R-ACPD on the amplitude of PF-mediated e.p.s.cs and on the holding current of Purkinje cells was observed in homozygous mutant mice ($n=8$), even when the concentration of 1S,3R-ACPD was raised to $500 \mu\text{M}$ (Fig. 4B). On the other hand, bath application of $100 \mu\text{M}$ L-2-amino-4-phosphonobutanoate (L-AP4), a selective agonist of certain presynaptic mGluRs¹, reversibly depressed PF-mediated e.p.s.cs ($71.54 \pm 4.20\%$) in homozygous mutant mice ($n=7$) while having no effect on the holding current of Purkinje cells (Fig. 4C).

In wild-type mice, as in rats^{10,25}, the pairing protocol induced a marked decrease in the amplitude of PF-mediated excitatory postsynaptic potentials (e.p.s.ps), which was apparent immediately after the period of pairing and lasted for the full 30 min that responses were followed, in 13 of 18 cells, from 8 mice. Thus, the mean amplitude of PF-mediated e.p.s.ps was $76.16 \pm 2.40\%$ of control (mean $\pm$ 1 s.d. of the mean) 20 min after the end of pairing. This LTD was accompanied by a decrease

of the initial slope of PF-mediated e.p.s.ps, without significant variation of their latency, of their time to peak, and of the input resistance of Purkinje cells (Fig. 5). In homozygous mutant mice, the same pairing protocol applied to 29 cells from 11 mice either did not induce any persistent depression of PF-mediated e.p.s.ps ($n=12$) or induced a small LTD of less than 15% ($n=11$). For the 20 cells that were held for at least 20 min after the pairing, the mean amplitude of PF-mediated e.p.s.ps was $91.72 \pm 2.35\%$ control ($P<0.01$ versus control; Student's *t*-test) (Fig. 5).

Electrophysiology of the hippocampus

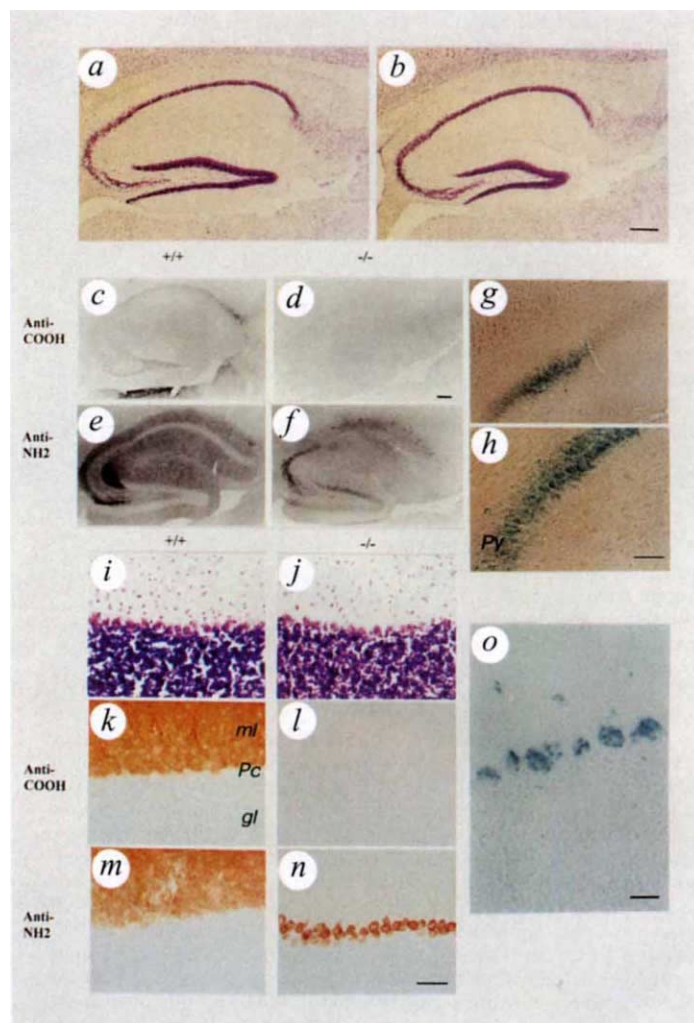
As the hippocampus is strongly implicated in spatial learning, we investigated electrophysiological properties of the hippocampus using standard slice techniques. According to all the criteria tested, the hippocampus of mGluR1 mutant mice appeared to be normal. Equivalent synaptic responses were evoked using similar stimulus intensities, in the Schaffer collateral–commissural pathway in area CA1, medial and lateral perforant pathways in the dentate gyrus and mossy fibre and associational pathways in area CA3. There was no apparent alteration in paired pulse depression in the medial perforant path or in paired-pulse facilitation in the other pathways (data not shown). Intracellular recordings from areas CA1 and CA3 revealed no differences; the resting membrane potentials (mV) and input resistances (M Ω) for area CA1 were -63 ± 1 and 40 ± 3 ($n=4$) compared with -64 ± 2 and 38 ± 3 (4) and for area CA3 were

FIG. 3 histological and immunocytochemical analysis of the hippocampus and of the cerebellum of wild type (+/+) and homozygous mutant (-/-) mice. Nissl staining (a, b) showed a normal appearance of the gross anatomical structure and staining with antisera against COOH-mGluR1 α (c, d) and against NH₂-mGluR1 (e, f) of hippocampal sagittal sections of wild-type (a, c, e) and of homozygous mutant mice (b, d, f). No immunoreactivity for COOH-mGluR1 α is detectable in homozygous mutant mice. NH₂-mGluR1 immunoreactivity, present in the neuropil of wild-type mice, vanishes in homozygous mutant mice, to appear in the perikarya of CA3 pyramidal cells, in granule cells of dentate gyrus and in some non-pyramidal neurons in the striatum oriens of CA1 (f). Low (g) and high (h) magnification photomicrographs of hippocampal sagittal sections of a homozygous mutant showing β -gal activity restricted to CA3 pyramidal cells. Nissl staining (i, j) showing a normal anatomical organization pattern and immunocytochemical reaction localization of COOH-mGluR1 α (k, l) and of NH₂-mGluR1 (m, n) through equivalent fields of the cerebellar cortex in wild-type and in homozygous mutant mice. Immunoreactivity for COOH-mGluR1 α present in the molecular layer of wild-type mice is not detectable in homozygous mutant mice. NH₂-mGluR1 reactivity appears to be restricted to cell bodies in mGluR1^{-/-} and in particular to Purkinje cell perikarya. o, Localization of β -gal activity in the cerebellum. All Purkinje cells express β -gal which is restricted to the soma, suggesting that the fusion protein mGluR1- β -gal is not transported any more to the dendrites. Calibration bar: 250 μ m (a, b); 170 μ m (c, d, e, f); 30 μ m (h); 50 μ m (i, j, k, l, m, n); 20 μ m (o). ml, molecular layer; gl, granular layer; Pc, Purkinje cells, Py, pyramidal cells.

METHODS. 8-week-old wild-type ($n=4$) and homozygous mutant ($n=3$) mice were used for immunocytochemical localization of mGluR1 receptors. Mice were killed under anaesthesia by intracardiac perfusion with saline followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Brains were dissected out, post-fixed overnight and maintained for 2 d in 10% sucrose in phosphate buffer at 4 °C. Sections (30 μ m) were cut with a cryostat, reacted with the first antibody and processed according to the indirect immunoperoxidase procedure using the biotin-streptavidin complex (Amersham) as described³⁸. Adjacent sections were collected and stained with cresyl violet. Antibodies were used at 0.2 μ g ml⁻¹ for anti-COOH-mGluR1 α and 1 μ g ml⁻¹ for anti-NH₂-mGluR1. Antisera preadsorbed with their respective peptide were used as control. X-gal staining was applied on 60- and 150- μ m-thick frozen sagittal sections of cerebellum and hippocampus from 3- and 8-week-old mice respectively, perfused and fixed with 4% paraformaldehyde.

-67 ± 2 and 42 ± 3 (6) compared with -66 ± 3 and 46 ± 4 (3) for wild-type and mutant mice, respectively. Finally, there were no significant differences in the actions of 1S,3R-ACPD in wild-type and in mutant mice. For example, the respective percentage depressions of field e.p.s.ps induced by 100 μ M 1S,3R-ACPD were 19 ± 6 (7) and 23 ± 6 (8) for area CA1, 39 ± 7 (6) and 44 ± 9 (4) for the associational pathway, and 48 ± 7 (7) and 56 ± 10 (4) for the mossy fibre pathway. Furthermore, spike frequency adaptation³ in response to depolarizing current was blocked by 1S,3R-ACPD in area CA1 (50–100 μ M) and area CA3 (10–20 μ M) to a similar extent in both wild-type (5) and mutant (6) mice. In addition, slow-onset potentiation of CA1 field e.p.s.ps³ was also seen upon wash-out of 1S,3R-ACPD in mutant mice (Z. A. Bortolotto, unpublished observations).

As LTP is an extensively investigated electrophysiological process which is often related to learning and memory, we determined the ability of five hippocampal pathways to undergo LTP in mGluR1 mutant mice. LTD and depotentiation are other forms of long-lasting synaptic plasticity in the hippocampus and so these were also investigated. In area CA1, LTP appeared normal, irrespective of whether it was induced using a θ -burst protocol (Fig. 6a) or by a single tetanus (Fig. 6b). In both mutant and control mice, low-frequency stimulation (900 shocks at 2 Hz, test intensity) failed to induce LTD (not shown) but led to equivalent depotentiation (Fig. 6a). There was no apparent difference between control and mutant mice in LTP induced in either the medial (Fig. 6c) or lateral (Fig. 6d) perforant



pathways. Furthermore, in area CA3, associational LTP was similar in control and in mutant mice (Fig. 6e). In contrast, mossy fibre LTP was greatly reduced in homozygous mutant mice (Fig. 6f): mean values after 60 min were $140 \pm 14\%$ ($n=10$) and $103 \pm 17\%$ (11) of control for wild-type and homozygous mutant mice, respectively ($P < 0.05$; Student's t -test). Expressed in terms of the number of animals displaying LTP (defined as a potentiation of at least 20% maintained for 60 min) the results were 8/10 and 1/11, respectively.

Discussion

Our results demonstrate that the absence of mGluR1 induces a severe motor deficit which may result, at least in part, from cerebellar dysfunction. In this context, it has been reported that cerebellar-ataxic spontaneous mutant mice such as *staggerer* (*sg*), *Purkinje-cell-degeneration* (*pcd*) and *Lurcher* (*Lc*) not only have abnormally few Purkinje cells, but also show no expression of mGluR1 in cerebellar cortex, although other glutamate receptors are still expressed²⁶.

Our physiological study also reports that the knockout of the mGluR1 gene does not lead to gross abnormalities of basic bioelectrical properties or of excitatory and inhibitory synaptic responses of Purkinje cells. The lack of effect of 1S,3R-ACPD on the excitability of homozygous mutant Purkinje cells is also consistent with both the complete deletion of mGluR1 receptors in these cells and with the postsynaptic origin of this current in normal cells²⁴. The lack of effect of 1S,3R-ACPD on PF-medi-

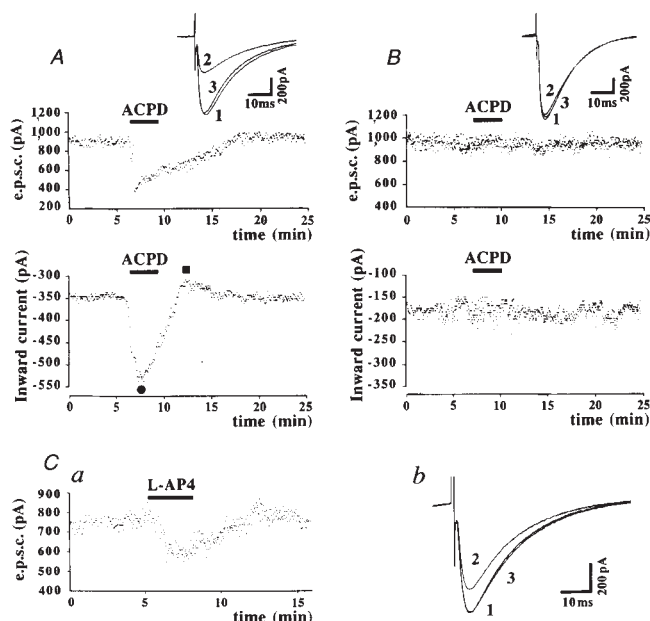


FIG. 4 Effects of mGluR agonists in wild-type and in mGluR1^{-/-} Purkinje cells. **A**, Bath application of 100 μ M 1S,3R-ACPD (horizontal bar) reversibly depressed the amplitude of PF-mediated e.p.s.cs and induced a marked inward current (filled circle) followed by an outward current (filled square) in a wild-type Purkinje cell. Insets in **A** and **B** are averaged e.p.s.cs before (1), during (2) and after (3) 1S,3R-ACPD application. **B**, None of these effects was observed in mGluR1^{-/-} Purkinje cells; insets as in **A**. **C**, **a**, In another mGluR1^{-/-} Purkinje cell, bath application of 100 μ M L-AP4 (horizontal bar) reversibly decreased the amplitude of PF-mediated e.p.s.cs. **b**, Averaged e.p.s.cs as in insets in **A** and **B**. **METHODS**. 200- μ m-thick sagittal slices were prepared from the cerebellum of 21-d to 4-month-old mutant and wild-type mice as described²⁵. The recording chamber was perfused with oxygenated Krebs solution containing (in mM): NaCl, 124; KCl, 3; NaHCO₃, 24; KH₂PO₄, 1.15; MgSO₄, 1.15; CaCl₂, 2; glucose, 10, pH 7.35 at 30 °C. Whole-cell recordings of Purkinje cells were performed at a somatic level, using an Axopatch-200 (Axon Instruments). The patch pipettes (2.5–4 M Ω) were filled with an internal solution containing (unless otherwise specified in the results) in mM: KCl, 140; NaCl, 8; HEPES, 10; EGTA, 0.5; ATP-Mg, 2; pH 7.35; 300 milliosmols. For HRP injection experiments, the internal solution was supplemented with 2.5% HRP. The enzyme was electrophoretically injected in the recorded cells with positive direct currents of 2 nA for 8 min. As reported previously^{10,25}, parallel fibres were stimulated at 0.4 Hz, except during pairing periods (see below), and PF-mediated e.p.s.cs were elicited on 10 mV hyperpolarizing voltage steps, thus allowing us to test passive properties of the recorded cells as well as the stability of access resistances. In pairing experiments (in the current-clamp mode), PF-mediated e.p.s.cs isolated in the presence of 10 μ M bicuculline in the bath were first evoked at 0.4 Hz in Purkinje cells maintained at -70 mV during a control period of at least 5 min to obtain baseline data. Then the pairing protocol consisted of 1 Hz stimulation of PFs for 1 min in conjunction with a steady firing of Ca²⁺ spikes elicited in Purkinje cells by a steady current passed through the recording electrode, and by depolarizing current pulses applied in conjunction with PF stimulations. After this pairing protocol, sequential testing of the input at 0.4 Hz was resumed immediately and Purkinje cell were maintained again at a holding potential of -70 mV. Furthermore, PF-mediated e.p.s.cs were elicited on hyperpolarizing responses induced by hyperpolarizing current pulses passed through the recording electrode (Fig. 5), allowing the testing of the input resistance of Purkinje cells and avoiding firing during PF-mediated e.p.s.cs.

ated e.p.s.cs in the mutant indicates that this effect is also normally mediated by mGluR1. The preservation of the depressant effects of L-AP4 in the mutant is consistent with this agonist acting on a different mGluR subtype, such as mGluR4 or mGluR7. In contrast to the cerebellum, no differences in the actions of 1S,3R-ACPD were seen in the hippocampus of wild-

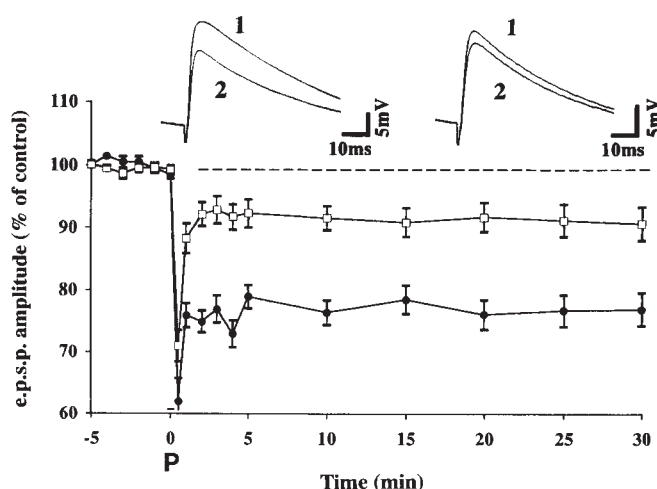


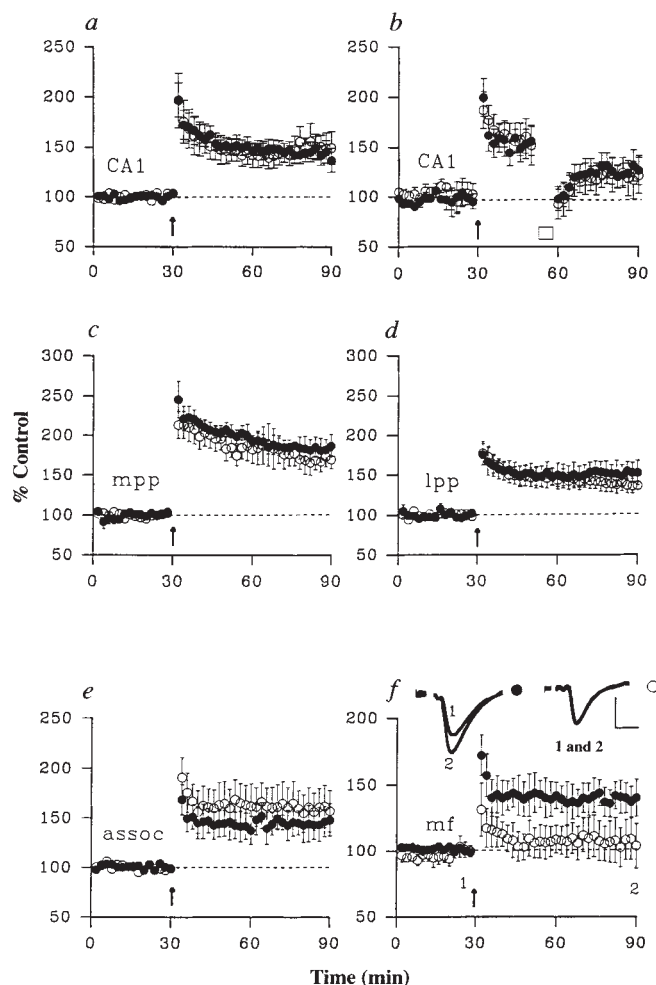
FIG. 5 LTD in control and in mGluR1^{-/-} mice Purkinje cells. Plots of amplitude of PF-mediated e.p.s.ps against time before and after a pairing protocol (P, indicated by heavy bar at $t=0$ min), in wild-type (filled circles) and in mGluR1^{-/-} Purkinje cells (empty squares) respectively. Each point is the mean $\pm$ s.e.m. of separate experiments in 18 wild-type (including 8 studied blind) and in 29 (including 10 studied blind) mGluR1^{-/-} Purkinje cells. Of the 18 wild-type cells, 6/8 that were studied blind and 7/10 that were not studied blind showed no recovery (amounting to more than 15%), so that a clearcut depression was already apparent 10 min after the end of pairing and either remained unchanged or still increased during the next 10 min before the amplitude of these responses also reached a plateau for the rest of the post-pairing period. In the 5 remaining cells, a small LTD ranging between 6 and 13%, depending on cells, was observed. Concerning the 29 mutant cells, 5/10 and 7/19 did not induce any persistent depression, 3/10 and 8/19 induced a depression amounting to less than 15% 10 min after the end of pairing and, finally, 2/10 and 4/19 displayed a depression in the range of that seen in most wild-type cells during this period in blind and non-blind experiments respectively. Insets on the left and on the right represent superimposed averaged e.p.s.ps recorded in a wild-type and in a mGluR1^{-/-} Purkinje cell respectively, during the pre-pairing period (1) and 15 min after the end of the pairing (2). Wild-type and mutant mice were treated comparably as far as housing and experimental conditions were concerned. Experiments on wild-type and homozygous mutant mice were intermingled and were done at the same time of day within 6 weeks.

type or mutant mice. This is probably because the actions are mediated, or in the mutant mouse have been compensated for, by other subtypes of mGluRs at which 1S,3R-ACPD is active.

Our results also indicate that, directly or indirectly, mGluR1 receptors are involved in the induction of LTD in the cerebellum. Previous pharmacological experiments^{10–13} have suggested a role for mGluRs in the induction of LTD, although the agents available could not distinguish the subtype involved. A specific mGluR1 antibody has been shown to eliminate the long-term depression of glutamate responses in cultured Purkinje cells normally induced by pairing depolarization with glutamate application²⁷. In mutant mice, a degree of LTD was still observed in some neurons, but this may underestimate the proportion of LTD mediated by mGluR1 in the wild type as a result of compensatory mechanisms in animals congenitally lacking mGluR1. Considered together, these results strongly implicate mGluR1 in cerebellar LTD.

A second major abnormality of the mutant mice is that they have severe learning impairments. As these animals have a greatly impaired mossy fibre LTP but in all other respects (including the ability to undergo LTP in four other hippocampal pathways) appear normal, it is possible that mossy fibre LTP is necessary for these learning tasks. Of course the possibility cannot be excluded that some other defect, which was not detected within the hippocampus, or which occurs outside it, is respon-

FIG. 6 Hippocampal synaptic plasticity in control and in mGluR1^{-/-} mice. Each graph plots the mean $\pm$ 1 s.e.m. of the slope of the field e.p.s.p., normalized with respect to the 10 min immediately preceding the tetanus, versus time for slices obtained from control (filled circles) and homozygous mutant (open circles) mice. *a* and *b*, Area CA1: *a*, no difference in θ -burst LTP between control ($n=5$) and mutant (7) mice. The induction protocol (presented at the time indicated by the arrow) comprised 5 bursts delivered with an interburst interval of 200 ms. Each burst comprised 4 stimuli at test intensity, delivered at 100 Hz. *b*, No difference in tetanus-induced LTP or depotentiation between control (8) and mutant (9) mice. The tetanus (arrow) comprised a single train (100 Hz, 1 s, test intensity) and the depotentiation induction protocol (delivered at the time indicated by the bar) comprised a period of low-frequency stimulation (2 Hz, 7.5 min, test intensity). *c* and *d*, Dentate gyrus, showing no difference in LTP in either the medial (mpp) (*c*) or lateral (lpp) (*d*) perforant pathways between control and mutant mice ($n=5$ in each case). In these experiments picrotoxin (100 μ M) was added to the perfusate and the tetanus comprised 100 Hz, 1 s, delivered at 10 times test intensity, to facilitate induction of LTP. Experiments on the medial and lateral perforant paths were performed on different slices and the pathways identified on the basis of the electrode placements, the shape of the synaptic responses and the response to paired-pulse stimulation³⁹. *e*, CA3 associational (assoc) pathway: showing no significant difference in LTP between control (10) and mutant (11) mice. *f*, CA3 mossy fibre (mf) pathway: showing a significant impairment in LTP in the mutant mice. In these experiments the tetanus (100 Hz, 1 s, test intensity) was delivered in the presence of 50 μ M AP5 to prevent the induction of NMDA-receptor-dependent LTP. (The associational LTP was examined in the same slices by delivering the tetanus (100 Hz, 1 s, test intensity) either before or following wash-out of AP5.) Insets show superimposed mossy fibre synaptic records obtained immediately before and 60 min following the tetanus for a control and mutant mouse, respectively. Calibration bar: 0.25 mV, 5 ms. METHODS. 400- μ m-thick slices were prepared from the hippocampus of 2–4-month-old mutant and littermate control mice using standard procedures. The recording chamber was perfused with oxygenated Krebs solution containing (in mM): NaCl, 124; KCl, 3; NaHCO₃, 26; NaH₂PO₄, 1.25; MgSO₄, 1; CaCl₂, 2; D-glucose, 10, at about 30 °C. Extracellular recordings were obtained from the dendritic fields of areas CA1, CA3 and the dentate gyrus using standard techniques. For each pathway studied, only one slice was used per animal. Experiments were done blind on slices from control and mutant mice and were randomly mixed.



sible for, or contributes to, the learning deficit. Irrespective of the actual lesion responsible for the learning deficit, these data provide evidence that mGluR1 is involved in learning. mGluR2 has also been implicated in olfactory memory²⁸. An mGluR antagonist, α -methyl-4-carboxyphenylglycine (MCPG), impairs spatial learning^{6,29}; these deficits could be caused by antagonism at any one or more of the subtypes of mGluR at which MCPG is active, which includes both mGluR1 and mGluR2 (ref. 30). The emerging role of mGluRs in learning is not surprising, given that these receptors provide the most direct means by which the synaptic release of glutamate can directly affect cell signalling systems.

Our results also increase understanding of the role of mGluRs in hippocampal LTP. Pharmacological studies have identified a role for MCPG-sensitive mGluRs in the hippocampus; in area CA1 they set a molecular switch which needs to be activated for the induction of LTP⁴. In the dentate gyrus they are necessary for the induction of LTP *in vivo*^{5,6}. Our findings that LTP in both these regions is normal suggests that the MCPG-sensitive mGluR(s) is not mGluR1. However, the possibility that other mGluRs have compensated for the deletion of mGluR1 and that this normally plays a role in LTP in these regions cannot be discounted; if compensation has occurred this would be more likely to apply to the dentate gyrus where mGluR1 is more highly expressed³¹. In area CA3, MCPG can³, but does not invariably³² block the induction of LTP. The present result that mossy fibre LTP is largely absent in the mGluR1^{-/-} mice suggests that, when effective, this action of MCPG is via mGluR1. The LTP seen occasionally in mutant mice, and the slightly more pro-

longed short-term potentiation, might reflect a compensatory mechanism or the existence of a parallel induction pathway that can operate under certain circumstances. Interestingly, despite the severe impairment in mossy fibre LTP, and the high expression of mGluR1 in the dendritic field of the associational projection, LTP in this pathway was not impaired in the mutant.

An interesting aspect of the role of mGluRs in synaptic plasticity is their relationship with the role of NMDA (*N*-methyl-D-aspartate) receptors. Both cerebellar LTD and mossy fibre LTP involve mGluR1 but do not require the activation of NMDA receptors. In contrast, the four hippocampal pathways that do not require mGluR1 for the induction of LTP all exhibit the NMDA-receptor-dependent form of LTP. In these pathways, LTP may require a different subtype of mGluR, such as mGluR5. These results could represent a general association between NMDA receptors and specific mGluR subtypes with respect to the induction of synaptic plasticity.

In conclusion, our results show that deletion of a single member of the mGluR gene family creates a striking phenotype which should be valuable for the study of synaptic plasticity. □

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LETTERS TO NATURE

Reassessment of millimetre-wave absorption coefficients in interstellar silicate grains

N. I. Agladze*, A. J. Sievers*, S. A. Jones†, J. M. Burlitch‡ & S. V. W. Beckwith‡

* Laboratory of Atomic and Solid State Physics and Center for Radiophysics and Space Research, Cornell University, Ithaca, New York 14853-2501, USA

† Department of Chemistry, Cornell University, Ithaca, New York 14853-1301, USA

‡ Max Planck Institut für Astronomie, 69117 Heidelberg, Germany

ALMOST all of the elements heavier than helium in the interstellar medium reside in small solid dust particles¹. Dust dominates the energy balance through its continuous absorption and emission of radiation. Its opacity provides the only direct means to assess the total mass in many interstellar regions: for example, in molecular clouds², in circumstellar disks undergoing planet formation^{3–5} and even in some galaxies⁶. But there are almost no laboratory measurements of small-particle opacities at low temperatures for any of the principal constituents of interstellar dust at the far-infrared and millimetre wavelengths at which most observations are made. Here we report a laboratory study of absorption by forsterite (Mg_2SiO_4), the pure magnesian form of the mineral olivine, which is a main constituent of interstellar dust. We have measured millimetre-wave absorption spectra of synthesized amorphous and crystalline forsterite powders between 3.5 and 15 cm^{-1} at temperatures below 30 K, the region most germane to estimates of interstellar dust mass. Our measurements indicate that the absorption per unit mass for this silicate is several times larger than that normally assumed in astronomical research^{7–10}. Estimates of the opacity of interstellar dust may have to be increased accordingly, causing the mass of interstellar particles derived from observations of the opacity to be lowered by a corresponding amount.

The exact composition of interstellar dust is still in dispute, and it probably varies from place to place, but silicate compounds are thought to dominate, through either olivine ($[\text{Fe}, \text{Mg}]_2\text{SiO}_4$) or a pyroxene (XS_2SiO_3 , where X is Fe or Mg). For our purposes, forsterite is essentially diamagnetic olivine. Only the low lying magnetic levels associated with the unfilled inner *d*-shell of Fe^{2+} are missing; otherwise, the dielectric properties of the two should be nearly the same. This means that amorphous olivine will absorb at least as strongly as amorphous forsterite because of the additional magnetic component.

The best estimates^{8–10} generally predict values for the mass-normalized absorption coefficient, κ , of interstellar particles: $\kappa \equiv \alpha/\rho \approx 0.3(\tilde{\nu}/10 \text{ cm}^{-1})^\beta \text{ cm}^2 \text{ g}^{-1}$ (particles only, no gas), where α is the absorption coefficient, ρ the density, $\tilde{\nu} = 1/\lambda$ the frequency and $\beta \approx 2$. So far there has been little consideration of the possibility that the long-wavelength absorption coefficient may depend on temperature.

We measured the absorption coefficients of amorphous and crystalline forsterite powders between 3.5 and 15 cm^{-1} in the laboratory at temperatures between 1.2 and 30 K. The forsterite

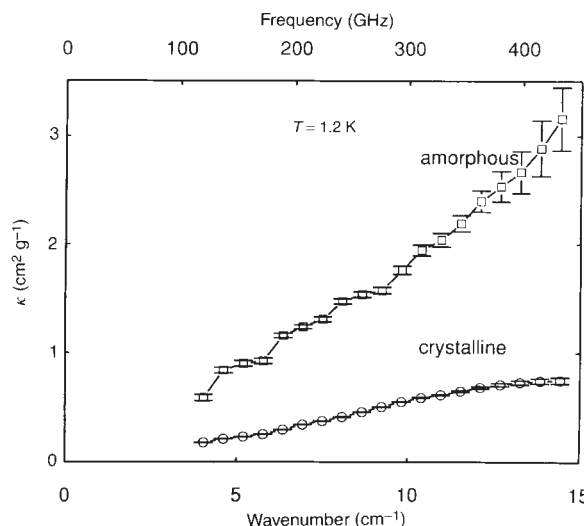


FIG. 1 Absorption coefficient per unit mass (κ) as a function of frequency for amorphous (top trace) and crystalline (lower trace) forsterite powders at 1.2 K. Both types of powders are measured in stainless-steel light-pipe cells capped by fused quartz vacuum windows. The two stainless-steel parts of a powder-filled cell are screwed together against an annealed oxygen-free high-conductivity (OFHC) copper O-ring in an evacuated and heated housing. During the evacuation process the entire assembly can be heated to 150 °C, as monitored by a chromel–alumel thermocouple, to activate the removal of adsorbed gases from the powder material before sealing. The length of each optical cell after sealing is 5 cm and the powder height is ~ 4 cm. One empty cell (with windows) is used as a reference. Random errors for the reference and the sample spectra produce the resulting r.m.s. deviations for κ shown in the figure. The additional systematic error of $\pm 0.1 \text{ cm}^2 \text{ g}^{-1}$ for the 'amorphous' curve and $\pm 0.02 \text{ cm}^2 \text{ g}^{-1}$ for the 'crystalline' curve reflect the slight change in detector sensitivity for the reference samples. Note that this systematic error (not shown in the figure) dominates over most of the frequency region shown.

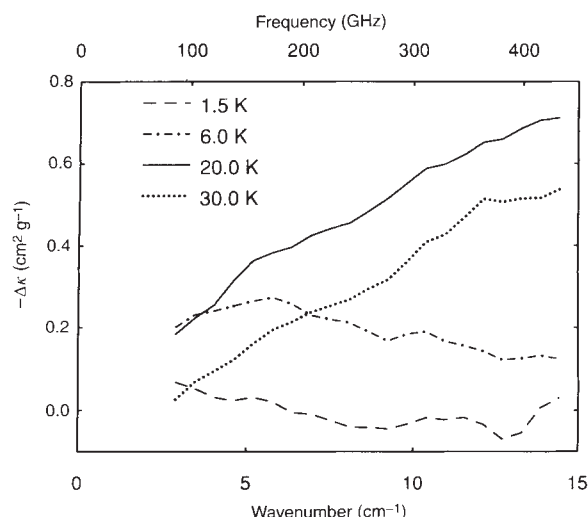


FIG. 2 Temperature dependence of the mass-normalized absorption coefficient difference for amorphous forsterite versus frequency. The ordinate is given by $-\Delta\kappa = [\kappa(T_r) - \kappa(T)]$, where $T_r = 1.2$ K. At these frequencies, the absorption coefficient of the pure fused silica millimetre-thick windows on the cell is so small that they appear completely transparent at all temperatures studied. The relative precision is higher here than in Fig. 1 because the sample position was not changed between temperature runs.

powders were synthesized by a H_2O_2 -assisted sol-gel reaction¹¹. Typical particle sizes of the crystalline powder measured with an optical microscope were $\sim 1 \mu\text{m}$.

Both types of powder were placed in stainless-steel light-pipe cells with fused-quartz windows attached by epoxy adhesive. The measured density of the crystalline powder was 0.97 g cm^{-3} ; the density of the amorphous powder was 0.11 g cm^{-3} . These values should be compared to the tabulated density of solid forsterite of 3.22 g cm^{-3} . This significant difference in densities occurs because the amorphous grains have an open fractal-like structure. Note that the effective volume fill fraction of the amorphous powder is small enough that the mass-normalized absorption coefficient can be compared directly with that determined for isolated grains.

The far-infrared spectra were measured with a lamellar Fourier spectrometer equipped with a liquid- ^3He -cooled germanium bolometer detector¹². The lamellar spectrometer is a two-arm Fourier-transform device but with the arms parallel, in contrast to the usual Michelson interferometer configuration.

Figure 1 shows the low-temperature behaviour of the mass-normalized absorption coefficient for forsterite powders between 3.5 and 15 cm^{-1} in the amorphous and crystalline states. The largest absorption over this frequency region is produced by the amorphous powder which was evacuated for 1 hour at room temperature before the cell was sealed. The low-temperature absorption coefficient is about three times greater for amorphous forsterite than for the crystalline powder. This extra dipole strength in the amorphous grain could come from local non-stoichiometry. The resulting extra absorption produced by charge fluctuations in a vibrational spectrum has been treated by Schlömann¹³. Note that the mass-normalized absorption coefficient of strongly absorbing soda-lime glass (Na_2O 17 wt%, CaO 9 wt%, SiO_2 72 wt%, traces < 2 wt%) is more than a factor of three smaller¹⁴ than for the amorphous forsterite shown in Fig. 1. At the lowest temperatures, the millimetre-wave absorption coefficient of soda-lime glass is dominated by two-level resonant absorption produced by a constant density of low-lying tunnelling states¹⁵; at higher temperatures, relaxation processes seem to control the absorption coefficient^{14,16}.

Because interstellar grains are probably disordered, an important question is whether or not disorder-related processes (such

as those in bulk glass) play a significant role in the far-infrared thermal emission from such sources. These temperature-dependent effects are not usually considered when extrapolating short-wavelength optical constants to longer wavelengths^{8,10}. It is therefore useful to determine whether similar effects occur in small particles of astronomically important materials. Mass estimates of interstellar particles depend entirely on these constants.

Our data for the amorphous powder can be fitted approximately with a power law, $\kappa(\omega) \approx \tilde{\nu}^\beta$, as is customary in astronomy with the power-law index, β , being 1.3 for the amorphous sample between 5 and 15 cm^{-1} . This value is substantially smaller than the value of 2 normally adopted for interstellar dust^{1,10} but very much in accord with values recently found for circumstellar particles^{3,17}.

For amorphous forsterite $\kappa = 1.8 \text{ cm}^2 \text{ g}^{-1}$ at 10 cm^{-1} . This value is approximately six times that calculated from shorter-wavelength extrapolations^{1,8-10} assuming $\beta = 2$. The coefficient is about three times smaller for crystalline material, and, of course, it is not yet known whether the iron-bearing species are significantly different. Nevertheless, these first measurements suggest that the low-temperature absorptivity of interstellar particles at these long wavelengths could be substantially larger than conventionally assumed.

The temperature dependence of the mass-normalized absorption coefficient is most easily analysed by examining spectra which represent the difference between two temperatures. Our results are presented as $-\Delta\kappa = \kappa(1.2 \text{ K}) - \kappa(T)$ versus frequency, where T is the absolute temperature in K. Figure 2 shows the temperature-dependent results for amorphous forsterite. When $\kappa(T)$ decreases above 1.2 K, $-\Delta\kappa$ is greater than zero. This 'bleaching' behaviour with increasing temperature is observed over most of the temperature range. At 30 K (Fig. 2, dotted curve), the trend is reversed, and the temperature-dependent absorption coefficient is larger than it is at 20 K. The bleaching behaviour with increasing temperature in the crystalline powder sample is about an order of magnitude smaller, although it is otherwise similar. For comparison, at 35 K, the absorption coefficient of bulk NaCl is less than $6 \times 10^{-3} \text{ cm}^2 \text{ g}^{-1}$ for frequencies below 10 cm^{-1} , and it decreases rapidly with decreasing temperature at all frequencies below the infrared-active vibrational mode¹⁸.

The investigation of the low-temperature bleaching effect in the millimetre-wave absorption of bulk glasses^{14,15} demonstrated

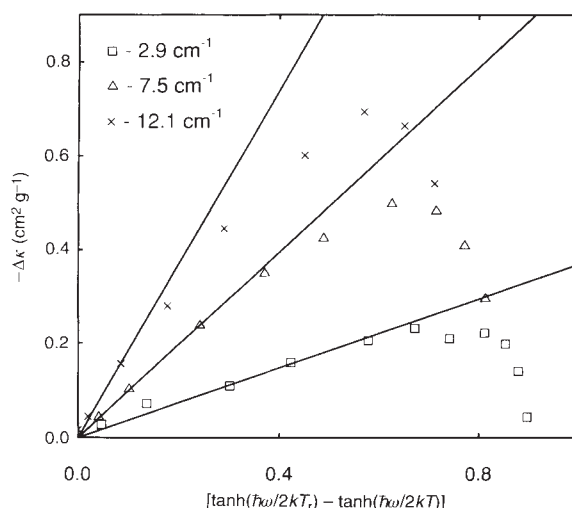


FIG. 3 Population dependence of the mass-normalized absorption coefficient difference for amorphous forsterite powder. The ordinate is the same as Fig. 2 and $T_r = 1.2$ K. Where the data follow the straight lines, two-level resonant absorption is dominant.

that a similar unusual temperature dependence could be associated with the $\tanh(\hbar\omega/2kT)$ population effect from the uniform distribution of two-level systems previously identified in the low-temperature specific heat of glasses¹⁹. Figure 3 shows a family of such temperature-dependent $\Delta\kappa$ curves for the amorphous powder for three different frequencies, plotted in such a way that if the two-level population effect is dominant, the temperature-dependent data at any fixed frequency should fall on a straight line going through the origin. Each $-\Delta\kappa$ set at fixed frequency came from temperature measurements at 1.2, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 11, 15, 20, 25 and 30 K. The data follow the temperature dependence expected for two-level systems up to a temperature of ~ 10 K, but additional absorption mechanisms such as phonon-assisted tunnelling and thermally activated transitions may become important at higher temperatures¹⁴. Our low-frequency temperature-dependent results appear very similar to those found for bulk glass.

Observations of thermal radiation from particles are converted to total particle mass by using an estimate of the temperature and the theoretical absorptivity of these constituents, dominated by the silicates². Neither the mix of temperatures nor the opacities are well known, but the temperatures can usually be derived from the shape of the spectra compared to (for example) Planck functions, whereas the opacity is assumed and therefore potentially contributes a larger uncertainty to the derived masses. The laboratory measurements yield surprisingly large values for the millimetre-wave absorption coefficients of small particles similar to interstellar grains. The presence of additional absorption mechanisms in amorphous material at long wavelengths beyond the asymptotic values extrapolated from short wavelengths¹⁰ indicates a revised value a factor of about six larger for the long-wavelength emissivity of solid interstellar material, assuming similar results are obtained for the other astrophysically important dust constituents. Such a revision will help resolve several astronomical problems, as well as being of general importance for all new estimates of the mass of interstellar material. For example, it lowers the derived masses of circumstellar disks^{20–22} in many cases so that the disks are less massive than their central stars; the disks would then be sufficiently stable to create planets. The slope of the frequency-dependent opacity coefficients is then in accord with that found for the disks and for large-scale distributions of dust in the Galactic plane^{23,24}. This revision will also change the overall calibration of the ratio of gas to dust mass in molecular clouds used routinely to assess the mass of regions of star formation². □

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Superconductivity in lanthanum nickel boro-nitride

R. J. Cava*, H. W. Zandbergen†, B. Batlogg*,
H. Eisaki‡, H. Takagi§, J. J. Krajewski*,
W. F. Peck Jr*, E. M. Gyorgy* & S. Uchida‡

* AT&T Bell Laboratories, Murray Hill, New Jersey 07974, USA

† National Centre for High Resolution Electron Microscopy,

Delft Institute of Technology, Delft, The Netherlands

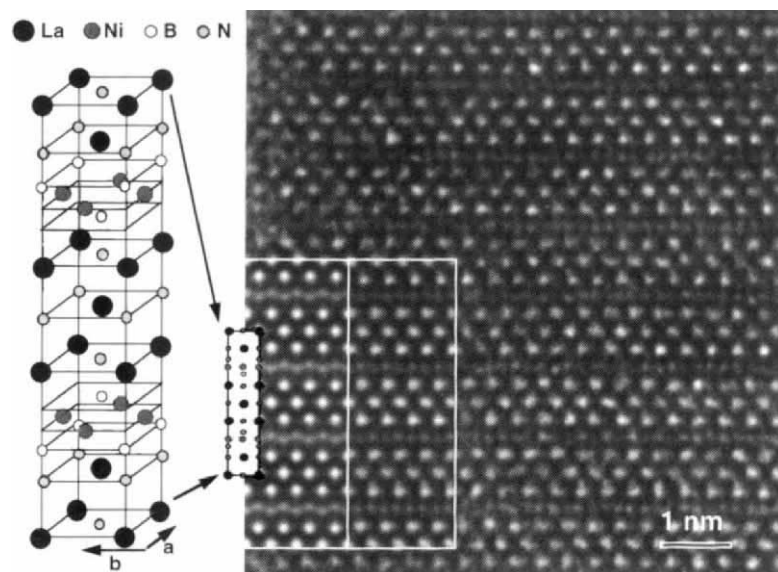
‡ Department of Applied Physics, University of Tokyo, Hongo, Tokyo, Japan

§ Institute for Solid State Physics, University of Tokyo, Roppongi, Tokyo, Japan

RESEARCH into intermetallic superconductors has recently been reinvigorated by the discovery of superconductivity at 23 K in a Y–Pd–B–C quaternary alloy¹ (tying with the long-standing intermetallic record held by Nb₃Ge (ref. 2)), and at lower temperatures in other quaternary boro-carbide alloys based on Ni, Pd and Pt^{3–6}. The crystal structure of these new intermetallic superconductors, typified by LuNi₂B₂C (ref. 7), consists of one square layer of rock-salt-type LnC (where Ln stands for a rare-earth element) alternating with one layer of transition-metal boride tetrahedra. Here we report superconductivity at 12–13 K in a new quaternary intermetallic system, lanthanum nickel boro-nitride. We have determined the formula and crystal structure of superconducting La₃Ni₂B₂N₃, and also of a related non-superconducting phase LaNiBN. The crystal structure of La₃Ni₂B₂N₃ is related to that of LuNi₂B₂C, but consists of three rock-salt-type LaN layers alternating with tetrahedral Ni₂B₂ layers, an arrangement considerably more two-dimensional than is found for the superconducting boro-carbides. These discoveries strongly suggest that a large number of unusual superconducting intermetallic phases are yet to be found, based on materials more complex than have previously been considered as candidates for superconductivity.

Samples were prepared by arc-melting and annealing. The starting materials were lanthanum metal shavings (99.9% purity), nickel powder (99.99%) and coarse boron powder (99.6%). LaN powder could also be employed as a starting material if care was taken to avoid significant air exposure. Nitrogen was introduced to the samples by arc-melting 0.5-g samples (three times, standard copper hearth) under N₂. In this process the arc-melted button absorbs nitrogen from the high-temperature nitrogen plasma in the electric arc. Although the amount of nitrogen incorporated in such a process may not necessarily be optimal, the samples were of better quality than those we obtained from initial experiments in sealed-tube reactions employing La, LaN, Ni and B powders, although that method might be worth further pursuit, as might other methods for synthesizing metal nitrides⁸. Lanthanum–nickel–boron intermetallic samples arc-melted under argon showed no superconductivity, and there are no elemental, binary or ternary superconductors involving these elements (or these elements in combination with nitrogen) with superconducting transition temperatures as high as we observed. Excellent-quality single-phase samples of La₃Ni₂B₂N₃ could be reproducibly synthesized by arc-melting La₃Ni₂B₂ (from the elements) under N₂ and annealing (tantalum-wrapped, in an evacuated quartz ampoule) for two or more nights at 1,050 °C. The non-superconducting phase LaNiBN is more difficult to synthesize because it is more refractory than La₃Ni₂B₂N₃, and there is nonequilibrium melting of samples during low-temperature anneals. Samples of LaNiBN with 90% purity, and nearly free of La₃Ni₂B₂N₃, were made by arc-melting LaNiB under N₂ and annealing at 950, 1,000, 1,050, 1,100, 1,150 and finally at 1,200 °C, with one-day soaks at each temperature. The samples of both phases, especially in powder form, are sensitive to atmospheric moisture; limited handling in air, however, is not a problem.

FIG. 1 Main figure, high-resolution electron microscope image of $\text{La}_3\text{Ni}_2\text{B}_2\text{N}_3$; La imaged as large white dots, Ni as small white dots; a axis horizontal, c axis vertical. Left inset, calculated image for structure with three LaN planes alternating with Ni_2B_2 tetrahedral layers; right inset, experimental image. A schematic view of the structure is shown on the extreme left, where La and Ni are large and small black circles, and B and N are small white and small shaded circles, respectively.



Superconductivity was observed for a wide variety of compositions in the La–Ni–B–N system. Powder X-ray diffraction studies of the samples after annealing indicated that the superconductivity was always associated with diffraction peaks characteristic of material of composition $\text{La}_3\text{Ni}_2\text{B}_2\text{N}_x$, which appeared to be single-phase. Specimens for electron microscopy study were obtained by crushing part of an arc-melted button of stoichiometry $\text{La}_3\text{Ni}_2\text{B}_2\text{N}_x$ in a glove box containing less than 1 p.p.m. H_2O and O_2 , suspending in sodium-dried hexane and putting a few drops on a copper grid covered with a carbon-coated holey film. The study showed the dominant presence of a lanthanum- and nickel-containing phase with a body-centred tetragonal unit cell of approximate dimensions $3.73 \times 20.67 \text{ \AA}$, and of small amounts of a lanthanum containing phase, which is a previously unreported non-superconducting lanthanum boronitride. This tetragonal cell provided an excellent description of the powder X-ray diffraction pattern of this and subsequent samples (which could be made free of the lanthanum boronitride second phase), from which more precise lattice parameters $a_0 = 3.721(2)$ and $c_0 = 20.516(5) \text{ \AA}$ were determined. High-resolution structure images were obtained with a Philips CM30ST electron microscope. Based on a comparison with the structures of $\text{LuNi}_2\text{B}_2\text{C}$ and LuNiBC (ref. 7), and image simulations, the crystal structure was found to consist of a series of

three square LaN layers in a rock-salt configuration, stacked with single tetrahedral Ni_2B_2 layers, yielding stoichiometry $\text{La}_3\text{Ni}_2\text{B}_2\text{N}_3$. Observed and calculated structure images, and a model of the structure are shown in Fig. 1. LaNiBN has a related structure, with two LaN layers stacked with Ni_2B_2 layers, isostructural with LuNiBC . Refinement of the unit-cell dimensions of LaNiBN from its powder diffraction pattern yielded (primitive tetragonal unit cell) $a_0 = 3.725(2)$, $c_0 = 7.590(4) \text{ \AA}$. The microscopy results will be described in detail elsewhere⁹. The nitrogen contents of several samples of $\text{La}_3\text{Ni}_2\text{B}_2\text{N}_x$ prepared as described were determined by commercial chemical analysis (LECO Laboratories, St Joseph, MI) to be $x = 2.7(1)$. This may indicate that the LaN layers can be sub-stoichiometric in nitrogen, or that the chemical analysis is not sufficiently sensitive to nitrogen content to allow an accurate stoichiometry determination. For the present purposes we refer to the superconductor by its ideal stoichiometry $\text{La}_3\text{Ni}_2\text{B}_2\text{N}_3$.

The magnetically measured superconducting transition (SQUID magnetometer, $H = 10 \text{ Oe}$) for a single-phase (no detectable impurities by powder X-ray diffraction) bulk polycrystalline sample of $\text{La}_3\text{Ni}_2\text{B}_2\text{N}_3$ is shown in Fig. 2. The zero-field-cooling data show a full shielding effect. The field-cooling data for this and other samples of $\text{La}_3\text{Ni}_2\text{B}_2\text{N}_3$ show a low Meissner effect, of the order of 5–10% (which is usual for this type of

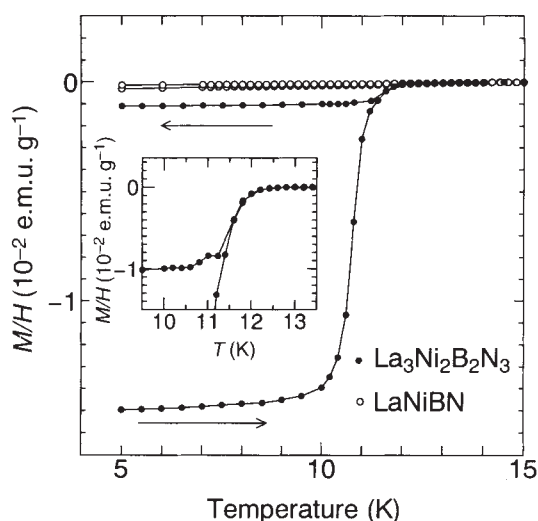


FIG. 2 Main figure, magnetization curves for a single-phase bulk polycrystalline sample of $\text{La}_3\text{Ni}_2\text{B}_2\text{N}_3$ annealed at $1,050^\circ\text{C}$ (filled circles) and for non-superconducting LaNiBN (open circles). Lower curves for each symbol, zero-field-cooling; upper curves, field-cooling ($H = 10 \text{ Oe}$). Inset, detail of region near T_c for $\text{La}_3\text{Ni}_2\text{B}_2\text{N}_3$.

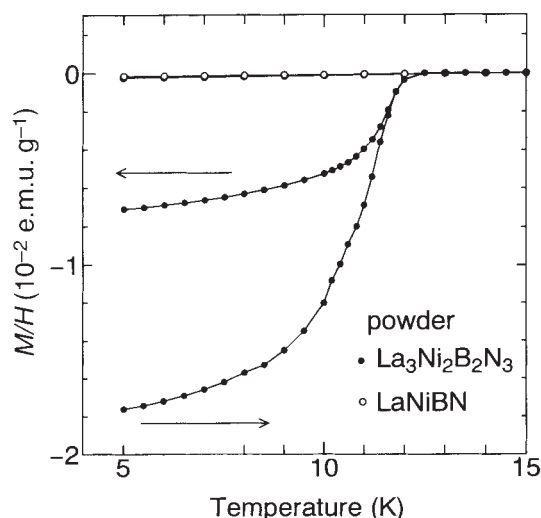


FIG. 3 Magnetically measured superconducting transition for single-phase $La_3Ni_2B_2N_3$ ground into a fine powder, using the same measuring conditions as Fig. 2. Also shown are data for $LaNiBN$ powder, showing the absence of bulk superconductivity above 4.2 K.

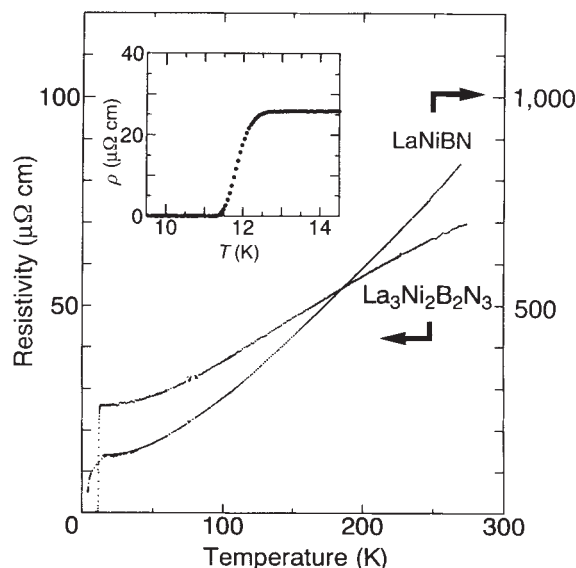


FIG. 4 Main figure, resistivity of polycrystalline sample of single-phase $La_3Ni_2B_2N_3$ annealed at 1,050 °C (left-hand vertical axis), and 90% purity $LaNiBN$ (right-hand vertical axis). Inset, detail of region near T_c for $La_3Ni_2B_2N_3$.

intermetallic sample), due to flux trapping. The Fig. 2 inset shows the region near the superconducting transition temperature (T_c) in detail, from which a T_c of 12.3 K can be determined. These data are for $La_3Ni_2B_2N_3$ annealed for two nights at 1,050 °C. Samples annealed at 1,100 °C show a transition temperature ~ 1 K higher. The origin of the T_c variation is not yet known, but may be related either to a variable nitrogen stoichiometry or to improved crystallinity: the widths of the powder-diffraction peaks decrease significantly, indicating improved sample uniformity, as the annealing temperature increases. Figure 2 also shows the temperature-dependent magnetization for a bulk polycrystalline sample of $LaNiBN$ of 90% phase purity. The data show that $LaNiBN$ is not a bulk superconductor at temperatures above 4.2 K. A screening signal of ~ 1 –2% of that expected for a fully superconducting sample is observed at 12.3 K, indicating the presence of a very small quantity (undetectable by X-ray diffraction) of $La_3Ni_2B_2N_3$.

Bulk polycrystalline samples of intermetallic superconductors typically show low Meissner effects, as seen in Fig. 2, owing to the absence of weak links, resulting in the trapping of magnetic flux unable to travel the macroscopic distances necessary to be expelled from the sample. To eliminate the possibility that an undetectably small amount of impurity phase coating the surfaces of grains of $La_3Ni_2B_2N_3$ was giving rise to the apparent superconductivity in that phase, we ground the bulk samples of high-purity $La_3Ni_2B_2N_3$ and $LaNiBN$ into fine powders and repeated the magnetic characterization of the superconducting transition. The results are shown in Fig. 3. With flux having to travel only on the order of micrometres to escape, a Meissner effect of $\sim 40\%$ is observed for $La_3Ni_2B_2N_3$, showing conclusively that it is a bulk superconductor. $LaNiBN$ in powder form shows no superconducting transition above 4.2 K. Because the samples in powder form are particularly susceptible to attack by atmospheric water vapour, we do not know whether the broadening of the super-

conducting transition observed in the powdered sample is intrinsic or due to partial decomposition.

Figure 4 shows the temperature-dependent resistivity for the bulk polycrystalline samples of $La_3Ni_2B_2N_3$ and $LaNiBN$. The normal-state resistivity at room temperature for $La_3Ni_2B_2N_3$, $\sim 70 \mu\Omega \text{ cm}^{-1}$, is larger than that observed in the superconducting boro-carbides⁴. This relatively high resistivity may be due to high-resistance grain boundaries formed during sample handling, as the boro-nitrides are sensitive to atmospheric moisture; further work is necessary to determine the intrinsic resistivity. The superconducting transition, shown in the inset, has an onset at ~ 12.3 K, a 10–90% width of ~ 0.8 K and zero resistance at ~ 11.4 K. $LaNiBN$ is also metallic, and shows a slight resistive transition due to the presence of small amounts of $La_3Ni_2B_2N_3$.

Finally, we note that superconducting transitions can be observed in samples made at a variety of different La:Ni:B ratios, when arc-melted under N_2 , which have shielding effects comparable to samples of stoichiometry $La_3Ni_2B_2N_3$. For samples of lower La to Ni ratio, the samples always contain detectable quantities of $La_3Ni_2B_2N_3$, especially when annealed at $\leq 1,100$ °C where $LaNiBN$ is poorly formed. For La-rich samples, $La_3Ni_2B_2N_3$ is again always present, but is mixed with various lanthanum boro-nitrides. No additional quaternary phases were formed. Therefore the presence of even 5–10% of $La_3Ni_2B_2N_3$ gives rise to a significant shielding signal.

This discovery of superconductivity in $La_3Ni_2B_2N_3$ demonstrates the existence of superconductivity in layered materials based on the stacking of Ni_2B_2 tetrahedral layers with single ($LuNi_2B_2C$) or triple ($La_3Ni_2B_2N_3$) MX rock-salt layers, establishing Ni_2B_2 layers as important building blocks for high-temperature intermetallic superconductivity in much the same way that CuO_2 layers are important for high- T_c oxides. $La_3Ni_2B_2N_3$ also considerably extends the chemical scope for further exploration by adding boro-nitrides to boro-carbides as promising quaternary systems for intermetallic superconductivity. □

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Physical and chemical evidence for metallofullerenes with metal atoms as part of the cage

David E. Clemmer, Joanna M. Hunter, Konstantin B. Shelimov & Martin F. Jarrold*

Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, USA

SINCE the discovery of fullerenes¹, efforts have been made to trap metal atoms inside fullerene cages², and both endohedral^{3,4} and exohedral^{5,6} metallofullerenes have been synthesized. There is, however, a third possibility: a 'networked' metallofullerene, where the metal atom is incorporated into the carbon cage. Here we report the results of experiments to study the structure and reactivity of gas-phase fullerenes doped with niobium (NbC_n^+ with $n = 28\text{--}50$). These experiments, which use injected-ion drift-tube techniques, indicate that for fullerenes containing an even number of carbon atoms the metal is endohedral, but for fullerenes with an odd number of carbon atoms, the niobium metal is bound as a part of the carbon cage. Thus, networked metallofullerenes appear to be a stable class of metallofullerene. We suggest that such metallofullerenes can form if the metal atom retains sufficient electron density to form several strong covalent metal-carbon bonds.

Although boron can be readily networked into fullerene cages⁷, evidence for a stable class of networked metallofullerenes is scant². It has been suggested that because the average nearest-neighbour C-C distance in a fullerene is only 1.44 Å, the small boron atom will be the only substitutional dopant, as found for graphite⁸. So far, the only evidence supporting the existence of networked metallofullerenes has been found for a few small LaC_n^+ ($n = 31, 33, 35$ and 37) clusters where the fullerene cage becomes too small to readily encapsulate the metal, thus squeezing it out of the cage⁹. Here we report physical and chemical studies of the structures of gas-phase NbC_n^+ ($n = 28\text{--}50$) clusters which show that networked metallofullerenes are stable for some metals. The networked structures are formed for clusters containing an odd number of carbon atoms, where the metal atom can occupy the defect site in the fullerene cage that results from the missing carbon atom.

Our injected-ion drift-tube apparatus has been described in detail previously¹⁰. NbC_n^+ ions generated by pulsed laser vaporization of a mixed NbC/graphite composite rod were size selected by a quadrupole mass spectrometer. Previous unsuccessful attempts at producing NbC_n^+ clusters have been interpreted as an indication that NbC_n^+ metallofullerenes are unstable¹¹. We were unable to observe NbC_n^+ clusters with a Nb:C ratio of 1:60, but they form readily when the ratio is 1:10. Pulses (25- or 50-μs) of mass-selected NbC_n^+ ions were injected (at an energy of 150 eV) into a drift tube containing helium. Drift-time distributions were collected under room temperature conditions (~300 K and ~5 torr), and with the drift tube cooled using liquid nitrogen (~77 K and ~2.1 torr). The drift time (the time required for the ions to travel across the drift tube) depends on the average collision cross-section, such that isomers with compact geometries have shorter drift times than ones with less compact geometries. This was first demonstrated for organic ions by Hagen using ion mobility spectrometry¹². von Helden *et al.* were the first to apply this idea to atomic-cluster ions using injected-ion drift-tube techniques^{13,14}. The room temperature conditions are used so that we can easily compare the mobilities measured here to ones measured previously^{9,13,14}. The cold con-

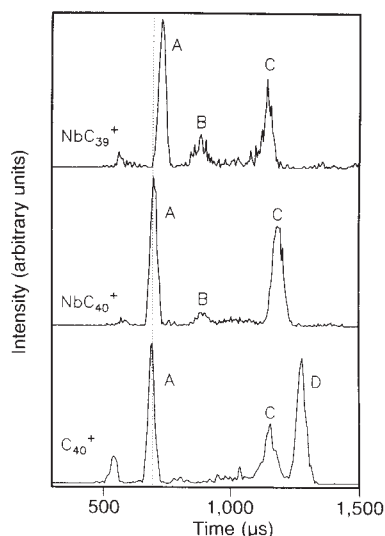


FIG. 1 Drift-time distributions recorded for C_{40}^+ (bottom), NbC_{40}^+ (middle) and NbC_{39}^+ (top) at 77 K and 2.070(±0.005) torr. The drift tube is 7.62 cm long and the drift field was 13.12 V cm⁻¹. Isomers A, B and C have been assigned to fullerene, graphitic sheets and bicyclic ring isomers, respectively (see text). Isomer D corresponds to a monocyclic carbon ring. The dotted line shows that the C_{40}^+ and NbC_{40}^+ fullerenes have nearly identical drift times, suggesting that the metal is inside the fullerene cage. The drift time for NbC_{39}^+ is substantially longer, suggesting that NbC_{39}^+ is not an endohedral metallofullerene.

ditions are used to enhance the resolution^{15,17}. On entering the drift tube, the ions experience a rapid transient heating cycle as their kinetic energy is thermalized by collisions with the buffer gas¹⁸. This causes some annealing but almost no dissociation (less than 10%). After leaving the drift tube, the ions are focused into a second quadrupole mass spectrometer that is used to monitor fragmentation and transmit only the ion of interest.

Figure 1 shows the drift-time distributions recorded for NbC_{39}^+ , NbC_{40}^+ and C_{40}^+ at 77 K. For C_{40}^+ , the peaks labelled A, C and D have been previously assigned to the fullerene, bicyclic ring and monocyclic ring isomer, respectively^{13,14}. Peak A for NbC_{40}^+ occurs at essentially the same time as peak A for C_{40}^+ , indicating that the former species is an endohedral metallofullerene. The most striking feature of the data in Fig. 1 is that the NbC_{39}^+ fullerene isomer has a longer drift time than the NbC_{40}^+ and C_{40}^+ fullerenes, even though this cluster contains fewer carbon atoms. This behaviour is observed for all of the neighbouring even-numbered (NbC_{2n}^+ or C_{2n}^+) and odd-numbered (NbC_{2n-1}^+) clusters above NbC_{36}^+ . For NbC_n^+ with $n \leq 36$, peak A always occurs at longer times than for the analogous C_n^+ cluster. Peaks B and C in the NbC_n^+ drift-time distributions are assigned to graphitic sheets and bicyclic rings containing niobium and will be discussed elsewhere (D.E.C. and M.F.J., manuscript in preparation). Monocyclic rings, an important isomer for pure carbon clusters (peak D), are not observed for NbC_n^+ clusters in this size range.

Figure 2 shows the inverse reduced (normalized to 273 K and 760 torr) mobilities for C_n^+ , LaC_n^+ and NbC_n^+ fullerenes determined from drift-time distributions collected at ~300 K and ~5 torr. As discussed previously⁹, the mobilities of LaC_{34}^+ , LaC_{36}^+ and larger clusters are essentially identical to those measured for the corresponding C_n^+ fullerenes, indicating that they are endohedral metallofullerenes. LaC_{37}^+ , LaC_{35}^+ and smaller LaC_n^+ fullerenes have inverse mobilities that are substantially larger than their corresponding C_n^+ fullerenes. For these clusters, calculated inverse mobilities for model metallofullerene structures show that the lanthanum is either exohedral or networked⁹. The measured mobilities for NbC_{37}^+ and smaller niobium metallofullerenes show similar behaviour to the small

* To whom correspondence should be addressed.

LaC_n^+ clusters, indicating a non-endohedral geometry due to the metal atom being squeezed out of the cage. But for the larger NbC_n^+ clusters, only those with an even number of carbon atoms form endohedral metallofullerenes. The larger odd-numbered NbC_n^+ clusters have inverse mobilities that are substantially greater than their C_n^+ analogues, indicating that the metal atom is not encapsulated within the carbon cage.

To test this idea further, we have studied the chemical reactivity of C_n^+ and NbC_n^+ clusters with O_2 and N_2 at 300 K. For these experiments, partial pressures of 0.002–0.008 torr of either O_2 or N_2 were added to the He buffer gas. Mass spectra and drift-time distributions were then collected to investigate the chemical reactivity of the NbC_n^+ clusters. All of the isomers displayed in Fig. 1 for pure C_n^+ clusters (over the entire 30–50 atom range) are unreactive towards both O_2 and N_2 at reagent partial pressures of up to 0.008 torr. Under these conditions, NbC_n^+ clusters react with oxygen to form $\text{NbC}_n(\text{O})_x^+$ ($x=1-5$); they react with nitrogen primarily by forming $\text{NbC}_n^+-(\text{N}_2)$ and to a much lesser degree $\text{NbC}_n^+-(\text{N}_2)_2$ (<10% of total product formation). Figure 3 shows the reactivity results recorded for NbC_{39}^+ and NbC_{40}^+ with N_2 at a partial pressure of ~ 0.002 torr. These data have been normalized and scaled by the measured fraction of the unreacted parent ions, such that the peaks in the drift-time distributions represent the abundance of each NbC_{39}^+ and NbC_{40}^+ isomer that remains after the clusters have passed through the drift tube. Clearly the NbC_{39}^+ fullerene isomer reacts with N_2 whereas the NbC_{40}^+ fullerene is essentially inert. When the same fraction of O_2 is added to the He buffer gas, no reaction is observed for the NbC_{40}^+ fullerene whereas the NbC_{39}^+ fullerene reacts away entirely. Results comparable to those for NbC_{40}^+ are observed for NbC_{38}^+ and larger NbC_{2n}^+ clusters. All NbC_{2n-1}^+ clusters, and NbC_{2n}^+ fullerenes that contain fewer than 38 carbons, show substantial reactivity towards both reagent gases, as was observed for NbC_{39}^+ . These results indicate that the Nb atom is responsible for the observed reactivity, and that for NbC_{2n-1}^+ fullerenes, the metal is accessible at the surface of the cage.

The results presented here show clearly that the niobium atom is trapped inside even-numbered carbon cages (having at least

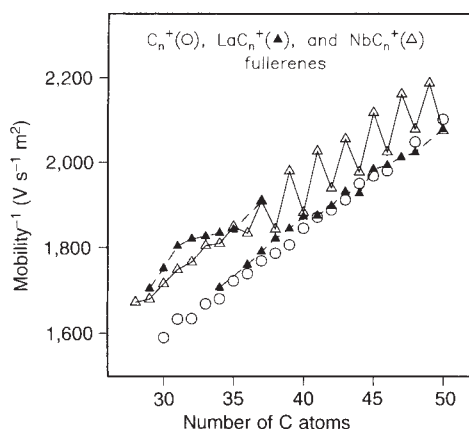


FIG. 2 Inverse reduced mobilities for the fullerene isomers (determined from data taken at ~ 300 K and ~ 5.0 torr) plotted against the number of carbon atoms in the cluster for C_n^+ (open circles), LaC_n^+ (solid triangles) and NbC_n^+ (open triangles). The inverse mobilities for LaC_{29}^+ – LaC_{35}^+ and LaC_{37}^+ are 9–12% larger than the inverse mobilities for the pure C_n^+ clusters; this indicates that lanthanum is attached to the outside surface of the fullerene. The mobilities for LaC_{36}^+ , LaC_{38}^+ and larger LaC_n^+ are essentially identical to those measured for C_n^+ , indicating that the metal is endohedral. In the niobium system, NbC_{38}^+ and larger NbC_{2n}^+ clusters have inverse mobilities that indicate that they are endohedral metallofullerenes. All of the NbC_{2n-1}^+ fullerenes, as well as NbC_{36}^+ and smaller NbC_{2n}^+ metallofullerenes, have mobilities that imply an isomer where the metal is attached to the outside surface of the fullerene.

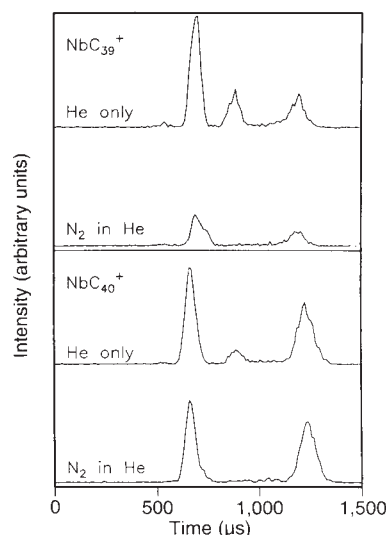


FIG. 3 Drift-time distributions for NbC_{39}^+ (top two traces) and NbC_{40}^+ (bottom two traces) in pure He and in a mixture of N_2 in He (see text). Isomer B, the graphitic sheet, reacts away completely for both NbC_{39}^+ and NbC_{40}^+ . Isomers A and C (the fullerene and bicyclic ring isomers, respectively) are substantially more reactive for NbC_{39}^+ than for NbC_{40}^+ . The low reactivity of the bicyclic ring (isomer C) for NbC_{2n}^+ is consistent with a geometry where the Nb atom is tetrahedrally coordinated by insertion into two orthogonal carbon rings (D.E.C. and M.F.J., unpublished results).

38 atoms) but resides at the surface of carbon clusters containing odd numbers of carbon atoms. The only reasonable explanation for the results for NbC_{2n-1}^+ fullerenes is that the metal can substitute for the missing carbon atom in an odd-numbered fullerene cage, stabilizing the resulting NbC_{2n-1}^+ metallofullerene. Our quantum-chemical calculations (using the Gaussian 92 program, Gaussian Inc., Pittsburgh) show that the resulting networked structures have a fairly distorted geometry—the metal atom sits outside the surface of the carbon shell—because the Nb–C bonds are significantly longer than C–C bonds (~ 2.0 Å for NbC compared to ~ 1.4 Å for carbon).

Insight into why niobium networks into large carbon cages, rather than being trapped inside (as observed for lanthanum) can be gained by considering the Nb^+ and La^+ ions. Nb^+ has four valence electrons and should bond strongly with the sp^2 -hybridized carbon atoms in the fullerene cage. La^+ has only two valence electrons and therefore cannot thoroughly satisfy the four-electron hole at the cage defect. Furthermore, electron paramagnetic resonance (EPR)¹⁹ and theoretical^{20,21} studies show that La^{3+} is found within fullerenes, further weakening the metal's ability to contribute covalently to the carbon framework. Thus the La^{3+} which resides on the inside of the fullerene cage is stabilized by ionic interactions with the negatively charged fullerene cage. On the other hand, the first and second ionization energies of Nb atoms (6.88 eV and 14.32 eV, respectively)²² are significantly larger than those of La atoms (5.577 eV and 11.06 eV)²². It therefore seems likely that Nb^+ will retain more of its valence electron density, enabling it to form covalent bonds with the four electrons at the defect site and network at the surfaces of even very large C_{2n-1}^+ cages. Indeed, our quantum-chemical calculations for networked NbC_{29}^+ and LaC_{29}^+ fullerenes determine a Mulliken charge of +1 on Nb and +2 on La, consistent with these simple arguments.

The preference of large even-numbered NbC_{2n}^+ clusters to form endohedral metallofullerenes can be understood by considering the energetic differences between carbon–carbon and metal–carbon covalent bonds. Thermochemistry for metal–carbon systems shows that the bond energies of even the strongest metal–

carbon single and double bonds (~ 2.5 eV and 4.1 eV, respectively)²³ are much weaker than carbon-carbon single and double bonds (~ 3.8 eV and 7.5 eV, respectively)²². Thus although the ionic interactions between lanthanum and the inside of a fullerene cage discussed above are probably weaker for niobium, the large energy difference between the formation of C-C and Nb-C bonds favours the all-carbon cage rather than the metal-substituted cage for NbC_{2n}⁺ metallofullerenes.

Our results show that networked metallofullerenes are a stable class of metallofullerene for some metals. In addition to the results for niobium described here, we have recently obtained preliminary evidence indicating that some of the larger zircon-

ium-containing fullerenes are networked. Because the metal atom is exposed in a networked metallofullerene, these species may possess catalytic properties as well as large dipoles and useful optical properties. They may self-assemble into novel layered materials. It should be possible to attach inorganic ligands during or immediately after production, which would not only provide a rational scheme for further stabilizing these species, but might also lead to extraction and purification procedures that use the metal-ligand chemistry. We are currently attempting to synthesize macroscopic quantities of these materials along these lines so that their properties can be examined in detail. □

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Effect of precipitation on the albedo susceptibility of clouds in the marine boundary layer

Robert Pincus & Marcia B. Baker

Geophysics Program AK-50, University of Washington, Seattle, Washington 98195, USA

TROPOSPHERIC aerosols are thought to have three important effects on the Earth's radiation budget: the direct radiative effect¹ (perturbation of clear-sky reflectivity), the indirect radiative effect² (modification of cloud albedo) and the effect on the hydrological cycle³ (modification of the vertical thickness and horizontal extent of clouds). The first two effects have been understood in principle for nearly 20 years, and quantitative estimates of their magnitudes have been provided by models and observations⁴. The third phenomenon, and its relation to the other two, has received far less attention. Previous work³ has shown, however, that increases in aerosol concentration may act to increase cloud albedo by increasing horizontal cloud fraction as well as cloud reflectivity. Here we use a simple model of the marine cloud-topped boundary layer to investigate the changes in cloud thickness and albedo that result from changes in precipitation as particle concentrations vary. We find that the sensitivity of layer cloud albedo to droplet number concentration (the albedo susceptibility) is increased by 50-200% when the dependence of cloud thickness on particle number is included. The results suggest that the response of cloud thickness to changes in aerosol particle concentration must be taken into account for accurate prediction of global albedo by climate models.

Aerosols affect cloud properties by acting as cloud condensation nuclei (CCN) upon which cloud droplets form. An increase in droplet number concentration N (cm^{-3}) at constant liquid-water mixing ratio q_l (kg water per kg air) results in a decrease in the average droplet radius and an increase in total droplet

surface area and cloud albedo A . The effect can be quite large—an order-of-magnitude change in N can induce relative changes in A as large as 30% (ref. 5). This process is often quantified in terms of albedo susceptibility⁶:

$$S_0 \equiv \left. \frac{\partial A}{\partial N} \right|_{q_l} \quad (1)$$

Measurements from satellites⁷ and aircraft⁸ of marine boundary-layer clouds around the world show that susceptibility varies widely under natural circumstances, and indicate that anthro-

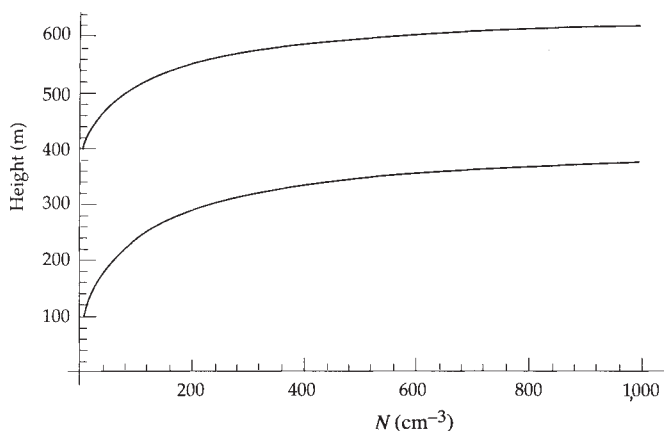


FIG. 1 Predicted equilibrium cloud thickness h (bottom trace) and cloud-top height z_i (top trace) as functions of droplet concentration N . Increases in N lead to decreases in the average cloud droplet radius and so to decreases in precipitation. This affects the boundary-layer energy budget, causing entrainment to increase and z_i to rise. Cloud thickness is most sensitive to changes in droplet concentrations when droplet concentrations are low. Droplet concentrations of 10 cm^{-3} have been observed in the Southern Hemisphere; concentrations of $1,000 \text{ cm}^{-3}$ represent highly polluted air from continental sources. The predictions arise from a mixed-layer model of the marine boundary layer which includes parametrizations of radiation and precipitation.

pogenic aerosols may modify cloud albedo even in remote locations⁸.

The role of CCN in controlling cloud vertical and horizontal extent is less clear. Albrecht³ considered the transition from fully overcast stratocumulus to partially cloudy cumulus clouds over the subtropical ocean, and linked marine boundary-layer cloud fraction to the efficiency of precipitation, which is dependent on droplet size⁹ and hence N . Albrecht did not report on changes in cloud thickness or albedo induced by changes in N . Boers and Mitchell¹⁰ recently suggested that changes in droplet number might affect boundary-layer dynamics and cloud liquid-water content by changing the amount of solar radiation absorbed by clouds. They did not quantify the degree of coupling, however, and they ignored precipitation.

Because of the importance of low-level oceanic clouds to global short-wave radiative forcing¹¹ we focus this study on stratocumulus clouds atop the marine boundary layer. Cloud albedo depends, in part, on the vertically integrated liquid-water content, which in these clouds is a simple function of geometric cloud thickness h (m). Because cloud-base height varies relatively little over the subtropical ocean, h is determined primarily by the cloud-top height z_i (m), which is established at the top of the boundary layer by a balance between entrainment and downward-directed large-scale subsidence. The process of precipitation, which depends indirectly on N , can modify h and thus A by affecting the rate of entrainment. Entrainment (the incorporation of overlying air into the boundary layer) is driven by the turbulence generated by cloud-top cooling, and the entrainment rate may be diminished by heating of the layer via absorption of solar radiation or through precipitation. For constant subsidence, then, h increases with long-wave cooling and decreases as radiative heating and/or precipitation increase.

Previous studies^{5,7,12} of the relationship between N and A have neglected the dependence of h on N . To investigate this connection we use a diurnally averaged steady-state mixed layer model of the cloud-topped marine boundary layer¹³. The boundary layer is represented as a horizontally uniform slab which is thoroughly mixed from the surface to the capping temperature inversion. The model equations describe the conservation of total-water mixing ratio and of static energy. Both are conserved during evaporation and condensation, but are modified by transfers of heat and moisture from the surface and across the cloud top, as well as by precipitation and radiative heating and cooling. Conservation of energy in a mixed layer requires a balance between cloud-top long-wave cooling and the heating due to surface fluxes of energy, absorption of short-wave radiation, condensation and entrainment of warm air across the inversion. We close the model by supposing that very little of the energy produced in the cloud layer is available to drive entrainment, a condition identified by Lilly¹⁴ and known as the 'minimum entrainment assumption'. We suppose that all CCN are activated, so that $N = N_{CCN}$ is constant with height, and link h , N and the average droplet radius r (μm) by assuming that the liquid water at cloud top (which increases linearly with h) is divided into N identical droplets per unit volume.

$$q_1 \propto Nr^3 \propto h \quad (2)$$

Although broadband solar absorption increases with r , the effect is small compared to the increase in absorption with cloud thickness. For fixed N , broadband short-wave absorption increases approximately linearly with cloud thickness¹⁵. We therefore approximate the diurnally averaged short-wave absorption SW (W m^{-2}) as

$$\text{SW} \propto h \quad (3)$$

The constant of proportionality in equation (3) is determined using a nominal droplet concentration of 100 cm^{-3} . This leads to a slight underestimate of solar absorption for large drops (small droplet concentrations) and overestimate for small drops which we examine below. Because relatively small amounts of

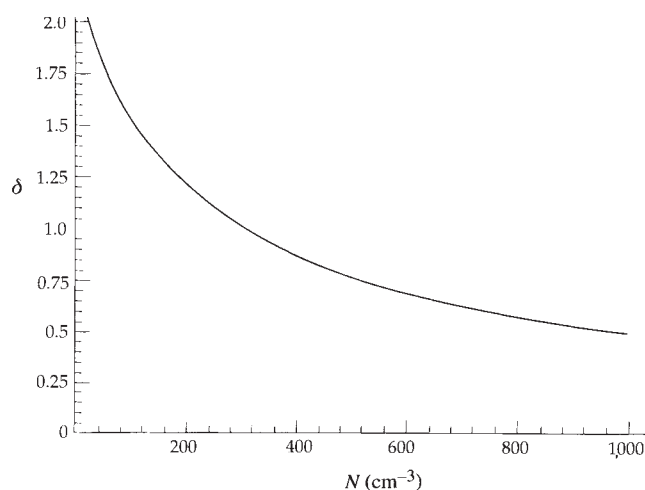


FIG. 2 Relative enhancement δ of albedo susceptibility S due to changes in cloud thickness as a function of droplet number concentration N . S has traditionally been calculated under the assumption that liquid water remains constant as N varies so that only changes in droplet size affect albedo and susceptibility; under these circumstances $S = S_0$. We use a mixed-layer model to calculate the relative effect of changes in cloud thickness h on S as compared to S_0 . The effect these changes have is largest where the sensitivity of h to N is greatest, but is of the same order of magnitude as S_0 at all droplet concentrations.

liquid water are opaque to infrared radiation, most marine boundary-layer clouds emit long-wave radiation as black bodies. For typical variations in cloud-top height the change in cloud-top temperature is negligible, so the long-wave radiative flux is fixed at a constant value.

Precipitation in stratocumulus clouds is primarily produced through the collision of small droplets with one another, a process known as autoconversion. An examination of autoconversion parametrizations^{16,18} and parametrizations describing precipitation in stratocumulus clouds¹⁹ show that all may be expressed in the general form:

$$P \propto h^m r^n \propto h^{m+n/3} N^{-n/3} \quad (4)$$

In the present work the constants in equation (4) are chosen to reproduce the parametrization of Turton and Nicholls¹⁸ ($m=1$, $n=3$). Drizzle evaporates at a constant rate below cloud base. It is the dependence of P on N and the subsequent effect of precipitation on the energy budget and entrainment rate that links cloud albedo and droplet number concentration in this model.

We solve the coupled algebraic equations describing a mixed layer in steady state¹³ using equations (3) and (4) to determine the solar absorption and precipitation fluxes, with numerical values of environmental parameters typical of the marine stratocumulus region off the coast of California²⁰. Figure 1 shows cloud thickness h and cloud-top height z_i as a function of droplet number concentration N in the range 10 – $1,000 \text{ cm}^{-3}$. Most of the variation in cloud thickness is due to changes in cloud-top height, although cloud-base height varies $\sim 60 \text{ m}$ across the same range. Cloud-top height increases with droplet concentration because, as we have noted, both droplet size and precipitation decrease as number concentration increases. If changes in droplet concentration did not affect the water and energy budgets through the production of precipitation and the absorption of solar radiation, h and z_i would remain constant at 420 and 650 m , respectively. Both quantities also depend on the environmental conditions, but the tendency of h and z_i to increase with N remains unchanged.

Cloud albedo depends parametrically on both h and N . Because these quantities are linked through precipitation and solar absorption, the sensitivity of cloud albedo to changes in

N has two terms:

$$\begin{aligned} S &\equiv \frac{dA}{dN} = \left. \frac{\partial A}{\partial N} \right|_{q_i} + \frac{\partial A}{\partial h} \frac{dh}{dN} \\ &= S_0 \left[1 + \frac{5N}{h} \frac{dh}{dN} \right] \\ &\equiv S_0 [1 + \delta] \end{aligned} \quad (5)$$

where albedo has been calculated using the two-stream approximation²¹. Ignored in equation (5) are variations in the asymmetry factor g (which describes the proportion of light scattered into the forward and backward directions by single scattering events) with r and hence N , but these variations are negligible given the range of droplet sizes considered. The sensitivity of cloud thickness to changes in droplet concentration dh/dN may be calculated from the set of model equations.

Figure 2 shows the relative enhancement δ of albedo susceptibility S due to changes in equilibrium cloud thickness as precipitation varies with N . Susceptibility S_0 varies with cloud optical depth, with a maximum⁶ at $A = 50\%$, or a cloud optical depth of ~ 13 . As Fig. 2 shows, changes in cloud thickness play a significant role in modulating cloud albedo susceptibility at all droplet concentrations and cloud thicknesses, although the enhancement is largest where dh/dN is greatest. At low droplet concentrations, changes in cloud thickness with droplet number are roughly twice as important in determining the sensitivity of albedo as are changes in droplet size with droplet number.

Neglecting the dependence of short-wave absorption on r in equation (3) causes an underestimate of solar absorption at small droplet concentrations. Including this effect would increase the contrast in cloud thickness with N , so that Fig. 2 represents a slight underestimate of the true sensitivity. More significant errors may arise because several important physical processes are not represented by the model. Evaporation of precipitation in the air between cloud base and the surface tends to cool and moisten this air relative to the cloud layer, inhibiting vertical mixing and promoting the formation of cumulus clouds²². If vertical motion is sufficiently reduced the mixed-layer assumption may be violated, a condition which may also be caused by the absorption of solar radiation. At extremely low droplet concentrations the cloud may become thin enough that the rate of long-wave cooling is diminished and mixing further reduced²⁸. Additionally, marine stratocumulus properties in nature are strongly modulated by the diurnal cycle of solar insolation, which drives daily variations in cloud thickness^{23–25} and precipitation. As the enhancement of albedo susceptibility predicted by this model is a first-order effect, further investigation with more complete models seems warranted.

Despite its simplicity, the model results are in qualitative agreement with observations of the increased albedo in boundary-layer clouds modified by ship exhaust ('ship tracks'). When clouds are thin and droplet concentrations low, the injection of aerosols into the marine boundary layer increases cloud liquid water and cloud albedo by suppressing precipitation²⁶. Thicker clouds with higher background albedo, on the other hand, exhibit changes in droplet radius but little change in albedo²⁷. The model predicts that cloud tops will be higher in ship tracks than in the surrounding environment; this is not evident in satellite observations of ship tracks²⁷, but may be masked by the natural variability in cloud-top height.

Albrecht³ noted that the effects of precipitation on horizontally averaged cloud fraction and cloud albedo act together to increase global cloud albedo as CCN concentrations increase. Our results suggest that, even in the absence of changes in cloud horizontal extent, precipitation processes and aerosol concentrations play an important role in determining layer cloud albedo through the modulation of cloud thickness. Previous work has shown that increases in aerosol concentrations increase global albedo by changing the clear-sky reflectivity and the size of cloud

droplets; the increase in cloud thickness with aerosol concentration discussed here adds to these effects, suggesting that aerosols may prove to be more important in determining planetary albedo than has been previously thought. $\square$

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A model study of the Atlantic thermohaline circulation during the last glacial maximum

Thierry Fichefet*, Stéphane Hovine* & Jean-Claude Duplessy†

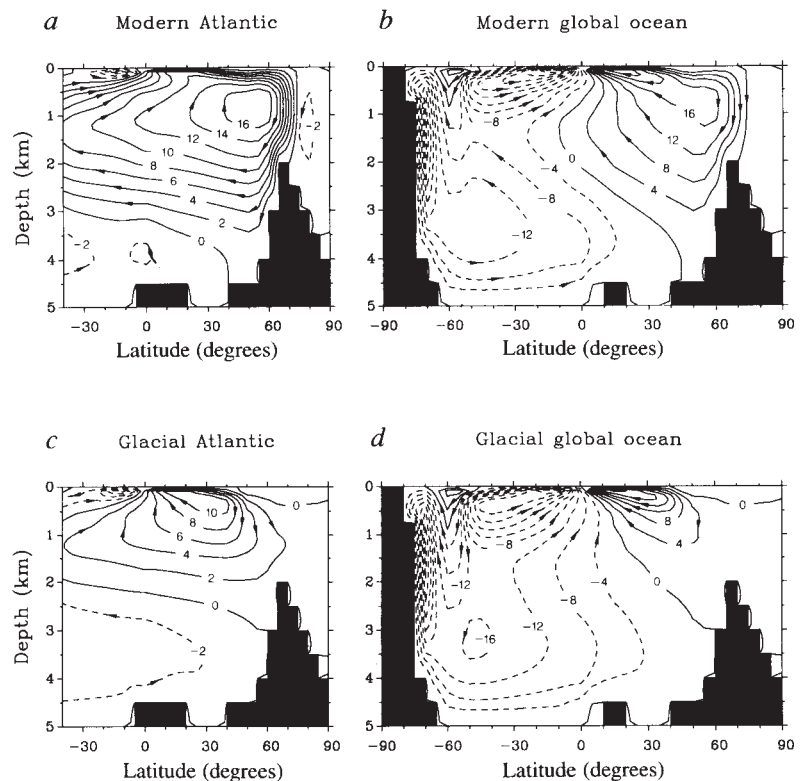
* Institut d'Astronomie et de Géophysique G. Lemaître, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium

† Centre des Faibles Radioactivités, Laboratoire mixte CNRS-CEA, F-91198 Gif sur Yvette Cedex, France

STABLE isotope measurements in deep-sea sediment cores have indicated that the Atlantic thermohaline circulation experienced significant changes during the last glacial maximum: the North Atlantic Deep Water (NADW) was shallower than today and the Antarctic Bottom Water (AABW) penetrated much farther north^{1–6}. Numerical ocean models have, so far, been unable to simulate these circulation changes realistically⁷. Here we show that a zonally averaged, three-basin ocean model, driven by glacial boundary conditions^{8–10}, reproduces the main trends of the geochemically constrained glacial Atlantic circulation. In addition, we provide quantitative estimates of the meridional water transport during glacial times. Our results suggest that the glacial production of AABW was slightly higher than at present, whereas that of NADW was reduced by $\sim 40\%$, resulting in an intermediate circulation cell which closed within the Atlantic basin. We also show that the strength of the Atlantic conveyor belt strongly depends on the surface density contrast between the high latitudes of the Northern and Southern hemispheres.

The model used here¹¹ is a global ocean circulation model consisting of three coupled, zonally averaged basins representing

FIG. 1 Contours of the annual mean meridional overturning stream-function in the Atlantic basin and in the three basins taken as a whole for the control run (a and b, respectively), and for the standard LGM experiment (c and d, respectively). The flow of sea water is parallel to the contours and is greatest where contour spacing is closest. Units are Sv ($1 \text{ Sv} = 10^6 \text{ m}^3 \text{ s}^{-1}$). In the control run, the atmosphere-to-ocean momentum flux over each basin is determined from monthly mean climatological wind-stress fields²¹. The surface fluxes of heat and fresh water are implied by restoring the temperature and salinity of the model uppermost level to the respective monthly and seasonal mean surface data of Levitus²² zonally averaged over the basin considered. Note that south of 70° S we use as restoring salinities the annual mean data at 100 m depth of Gordon and Molinelli²³ to avoid the bias of Levitus salinities there towards summer observations^{19,24}.



the Atlantic and Eurasian Arctic, the Pacific and Canadian Arctic and the Indian Ocean. (For convenience, the first two basins will be referred to hereafter as Atlantic and Pacific, respectively.) Each basin has its own bathymetry and a latitude-dependent angular width. The model dynamics is based on the zonally averaged form of the primitive equations for a hydrostatic, Boussinesq ocean with a rigid lid. The east-west pressure difference which arises on averaging the momentum equations is taken to be proportional to the meridional pressure gradient^{12,13}. The model also includes conservation equations of mass, heat and salt, and has a nonlinear equation of state. Lateral exchanges of heat and salt between the basins are permitted between 85° and 90° N , and between 40° and 65° S . The latitudinal resolution is 5° , and the vertical is resolved by 19 levels.

Two experiments are conducted with this model: a control run with present-day surface boundary conditions, and a run with conditions that prevailed during the last glacial maximum (LGM). In both cases, the model is integrated for 10,000 years under restoring boundary conditions from a state of rest with uniform temperature and salinity. The acceleration techniques of Bryan¹⁴ are employed during the first 2,000 years to speed up the convergence towards equilibrium.

The boundary conditions for the LGM experiment are obtained by adding anomalies of wind stress, temperature and salinity to the modern forcing fields. The wind-stress anomalies are derived from the ice-age response of an atmospheric general circulation model⁸. Regarding the anomalies of relaxation temperature, their values are deduced from the differences of sea surface temperature in February and August between the LGM and the present time⁹. The anomalies of relaxation salinity prescribed north of 40° S in the Atlantic originate from the reconstruction¹⁰ of the glacial-interglacial changes of summer surface salinity in this sector. The same anomalies are used north of 70° N in the Pacific basin. Elsewhere, we apply a salinity anomaly of +1 part per thousand (p.p.t.), which corresponds to the global salinity increase due to the glacial sea-level reduction¹⁵. It should be noted that we (J.-C.D *et al.*, manuscript in preparation) have found salinity modifications of this magni-

tude in the glacial Southern Ocean. The Bering Strait is closed in this experiment in accordance with glacial topography.

Figure 1a displays the annual mean meridional overturning stream-function in the Atlantic basin for the last year of the control run. Deep water is formed between 60° and 70° N at a rate of $\sim 17 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ (17 sverdrups, Sv). About 9 Sv of this water recirculate within the basin, the remainder exiting into the southern connection zone at depths of 1,500–3,000 m. About 30 Sv of AABW are created off the Antarctic continent (Fig. 1b). Of this water, only 2 Sv enter the Atlantic basin (Fig. 1a). Below 1,500 m, deep water penetrates into the Indian and Pacific basins at rates of $\sim 6 \text{ Sv}$ and $\sim 12 \text{ Sv}$, respectively (see Fig. 3 in ref. 11). This water then upwells roughly uniformly over the regions north of 40° S , and finally returns to the Atlantic basin via the southern connection area as intermediate- and upper-water masses (Fig. 1a). This circulation pattern is consistent with the conveyor-belt picture suggested by Gordon¹⁶. Superimposed on this thermohaline overturning are intense wind-driven cells in the upper thermocline.

One of the most prominent effects produced by the inclusion of glacial boundary conditions in the model is the cessation of deep-water formation in the high-latitude North Atlantic (Fig. 1c), which follows from the stratification due to the lower relaxation salinities there (Fig. 3a). Around 50° N , the surface-water salinity is sufficiently high to permit deep convection to a maximum depth of 2,500 m as a source of glacial NADW (Fig. 1c). This gives rise to a thermohaline overturning of moderate strength (the maximum value of the stream function amounts to $\sim 11 \text{ Sv}$) which is essentially confined north of 40° S (only 1 Sv of glacial NADW escapes into the southern connection zone). Below 2,500 m, most of the Atlantic basin is filled with AABW, so that the relative contribution of water from the Southern Hemisphere as compared to that from the Northern Hemisphere is much greater than in the control case. All these features are consistent with the reconstructions of the Atlantic circulation during glacial times derived from measurements of cadmium-to-calcium ratios and carbon-isotope ratios in benthic foraminifera shells¹⁻⁶. Moreover, it is noteworthy that the

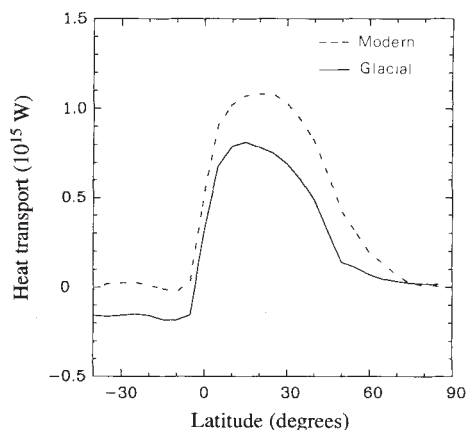


FIG. 2 Annual mean meridional heat transport in the Atlantic basin for the control run with present-day boundary conditions (dashed line) and the standard LGM experiment (solid line). Positive values indicate northward transport.

latitudes where the glacial NADW is produced in the model fully match the density-based model of deep-water formation by Labeyrie *et al.*¹⁷. Oppo and Lehman⁵ speculated that the NADW circulation may have been vigorous during the LGM because of the lack of carbon-isotope contrast at intermediate depths in the glacial North Atlantic. This is not supported by our simulation which shows a marked weakening of the conveyor. A detailed comparison of Fig. 1*d* and *b* indicates that the intensity of the thermohaline cell in the immediate vicinity of Antarctica increases by $\sim 10\%$ in the LGM experiment. This in turn slightly enhances the northward flow of bottom water in the three ocean basins. Apart from this stronger inflow of AABW, the Indian and Pacific basins exhibit no noticeable change of circulation. This behaviour needs, however, to be confirmed by performing additional experiments with a more reliable saline forcing in these basins.

Compared to the control run, the temperature of the deep Atlantic is reduced by 2–3 °C, in agreement with oxygen-isotope estimates¹⁸. This cooling is primarily caused by the replacement of the relatively warm modern NADW by cold AABW. Figure 2 illustrates the changes of meridional heat transport in the Atlantic basin induced by the use of glacial boundary conditions. Heat is transported northwards at almost all latitudes for the present climate. In the LGM case, as a consequence of the weakening of the thermohaline circulation, there is a reduced northward heat transport in the Northern Hemisphere and a southward heat transport in the Southern Hemisphere. North of 45° N, the transport of heat is reduced to nearly zero owing to the southward shift of the polar thermal front⁹.

Like all the palaeoclimatic reconstructions based on the oxygen isotopic composition of individual foraminiferal species, the precision of the salinity reconstruction proposed by Duplessy *et al.*¹⁰ is limited by a number of uncertainties. There is also some uncertainty about the salinity anomaly prescribed in the Southern Ocean area. To evaluate the sensitivity of the simulated glacial Atlantic circulation to inaccuracies of the saline forcing, a series of four experiments were carried out in which the standard anomalies of relaxation salinity are increased or decreased by 0.4 p.p.t. either north of 40° N in the Atlantic and north of 70° N in the Pacific, or south of 45° S in the three ocean basins (Fig. 3*a*). The experimental design is identical to that of the two experiments discussed above.

Figure 3*b* shows that the strength of the Atlantic overturning is governed by the density contrast between the regions where deep convection takes place. Decreasing the salinity anomalies by 0.4 p.p.t. in the north, or increasing them by the same amount in the south, leads to a significant weakening of the thermohaline

overturning and to a more pronounced intrusion of AABW. In both cases, the southern boundary of the conveyor lies at $\sim 10^\circ$ S. When the salinity anomalies are perturbed by +0.4 p.p.t. in the north or by -0.4 p.p.t. in the south, the overturning intensifies dramatically. The resulting circulations resemble that of the control run with present-day boundary conditions, except that deep water forms around 50° N and that the northward inflow of AABW is noticeably weaker. North of 10° N, the entire deep Atlantic is occupied by NADW in these two cases, which is inconsistent with geological evidence. Despite the relatively high sensitivity of the modelled glacial circulation to possible errors in the saline forcing, two features of the standard LGM experiment appear robust: (1) the existence of a glacial Atlantic conveyor belt, and (2) the absence of deep-water production north of 55° N.

Our study represents a first attempt to reconstruct the glacial Atlantic circulation with a dynamic ocean model. Although the model we use is idealized and so should be viewed as process-oriented rather than predictive, the results obtained here provide further evidence that a conveyor-type circulation was active in the Atlantic Ocean during the last ice age. However, the size of this conveyor must have been smaller than that of the modern Atlantic conveyor. More important, the overturning cell seems to have closed within the Atlantic basin, resulting in a vanishing

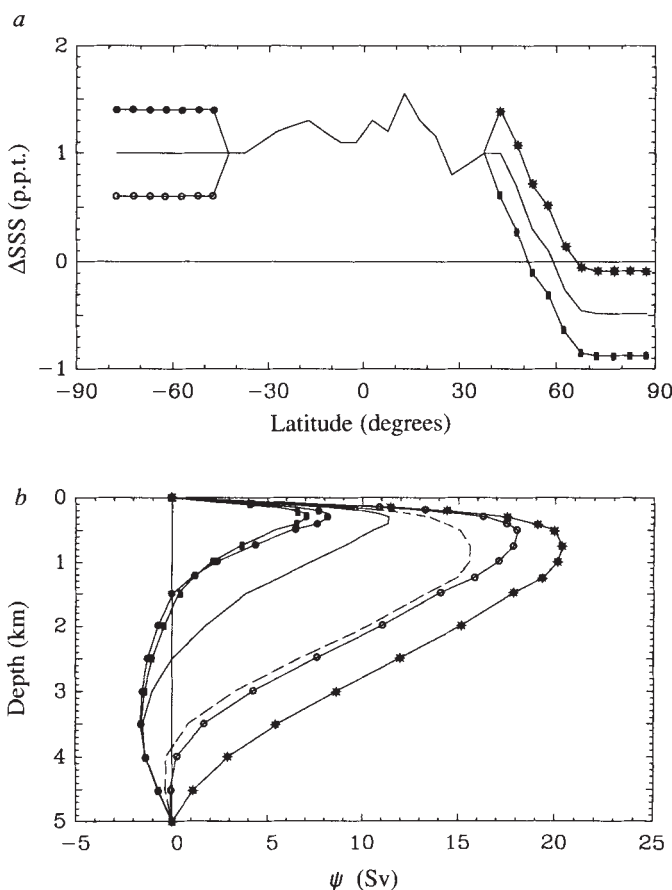


FIG. 3 *a*, Latitudinal profile in the Atlantic basin of the anomaly of relaxation salinity (ΔSSS) prescribed in the standard LGM experiment (solid line, no symbols) and in the four sensitivity experiments (solid lines with symbols). The anomalies are relative to the relaxation salinities of the control run with present-day boundary conditions. *b*, Vertical profile of the annual mean meridional overturning stream-function (ψ) at 30° N in the Atlantic basin for the control run (dashed line), the standard LGM experiment (solid line) and the four sensitivity experiments (solid lines with symbols).

contribution of glacial NADW to the other basins. Our results also point to the relatively saline surface waters at $\sim 50^\circ\text{N}$ as a probable source for a significant portion of the glacial NADW. The region of the Greenland–Norwegian seas was too low in salinity to be a sink for surface water. Another finding, which confirms the results of earlier studies^{19,20}, is that the intensity of the Atlantic thermohaline overturning is controlled by the density difference between surface waters in the northern North Atlantic and in the vicinity of the Antarctic continent. This emphasizes the need for reliable palaeoclimatic reconstructions of sea surface temperature and salinity over the whole world ocean. We note that Rahmstorf (ref. 25) has also recently attempted to model glacial thermohaline circulation in the North Atlantic. □

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Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes

Gilbert Cabana & Joseph B. Rasmussen

Department of Biology, McGill University,
1205 Avenue Docteur Penfield, Montreal H3A 1B1, Canada

THE nitrogen pools of animals are enriched in ^{15}N relative to their food¹, with the top predators having the highest concentrations of this stable isotope². The use of $\delta^{15}\text{N}$ to indicate trophic position depends on the degree to which it reflects variation in the underlying food-web structure, rather than variable fractionation along the food chain. Here we compare adult lake trout, a top pelagic predator, from a series of lakes, and find that $\delta^{15}\text{N}$ values vary from 7.5 to 17.5‰, a surprisingly wide range for one species. The length of the food chain can explain this variation, supporting the idea that $\delta^{15}\text{N}$ is a food-web descriptor. Food-chain length was measured by the presence or absence of two intermediate trophic levels, pelagic forage fish and the macrozooplankton, *Mysis relicta*, each of which when present contributes about three $\delta^{15}\text{N}$ units to the trout signature. We find that $\delta^{15}\text{N}$ can be used as a continuous, integrative measure of trophic position, which is supported by its correlation to mercury levels in lake trout.

A general approach to the measurement of food-web processes has been suggested by studies of stable nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$) of organisms and their food sources^{2,3}. In laboratory experiments, the enrichment in $\delta^{15}\text{N}$ ($\delta^{15}\text{N} = ([^{15}\text{N}/^{14}\text{N}_{\text{sample}} / ^{15}\text{N}/^{14}\text{N}_{\text{standard}}] - 1) \times 1,000$), where atmospheric nitrogen is the reference material, in animals relative to their diet is on average +3.4‰ for a wide variety of taxa¹. In field studies of aquatic food chains leading to different top predators, $\delta^{15}\text{N}$ increases from primary producer to top-level consumers^{4–7}. Variation in the $\delta^{15}\text{N}$ signature of primary producers at the base of the food chain^{8–11} and in the fractionation along the food chain can sometimes produce significant variation even within the same species of top predator^{10,11}, and thus the validity of $\delta^{15}\text{N}$ as a measure of trophic position has been questioned². Our ability to assess the utility of $\delta^{15}\text{N}$ as a food-web descriptor depends partly on the accumulation of a larger

database for comparative work, but also, and more importantly, on comparisons involving sites known to differ greatly in food-web structure.

We report here a wide variation in mean $\delta^{15}\text{N}$ (7.5–17.5‰) of adult lake trout from 24 Canadian shield lakes. Analysis of variance showed that over 90% of this variation was attributable to among-lake effects. Such a wide range in $\delta^{15}\text{N}$ across lakes could appear to invalidate $\delta^{15}\text{N}$ as a trophic indicator unless the trophic position of lake trout could be shown to vary by several trophic levels across these lakes, and to covary with $\delta^{15}\text{N}$.

Owing to limited postglacial dispersal¹² and anthropogenic introductions^{13–15}, the length of the pelagic food chain leading to lake trout is highly variable, with some important functional groups such as pelagic forage fish and the crustacean *Mysis relicta* being absent in many lakes. In lakes with the shortest pelagic food chains (class I lakes), where *Mysis* and pelagic forage fish are absent, adult trout feed predominantly on zoo-

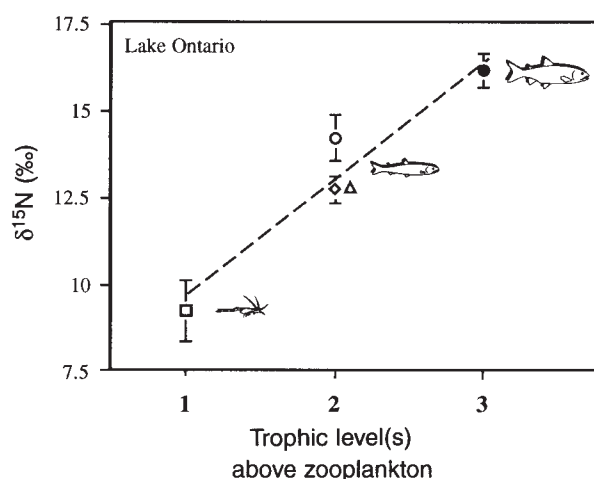


FIG. 1 Mean ($\pm$ s.e.) $\delta^{15}\text{N}$ for components of the Lake Ontario pelagic food chain. *Mysis relicta*, pelagic forage fish (smelt, alewife, sculpin) and lake trout are represented by, respectively, square, circle, diamond, triangle and filled circle. The least-square regression equation $\delta^{15}\text{N} = 3.46L_t + 6.11$ (adj. $r^2 = 0.91$, $P < 0.01$, $n = 5$), where L_t represents the hypothesized number of trophic levels above zooplankton and is shown as a broken line. Samples were collected by M. Servos and R. Kiriluk (Department of Fisheries and Oceans (DFO), Canada).

plankton and benthic organisms¹⁶. Trout feed mainly on pelagic forage fish when these are present (classes 2 and 3)^{16,17}. These pelagic prey in turn feed predominantly either on zooplankton or on the larger *Mysis*, when this last species is present (class 3)^{15,17}. Finally, *Mysis* prey on zooplankton and some large phytoplankton^{13,14}. We have shown that this three-step biogeographic food-chain model predicts the levels of biomagnifying persistent contaminants such as polychlorinated biphenyls and mercury in lake trout^{18,19}.

In Lake Ontario, a class 3 lake, $\delta^{15}\text{N}$ increased by an average of +3.5‰ from *Mysis*, through pelagic forage fish, to lake trout (Fig. 1). This intra-lake study suggested that the absence of

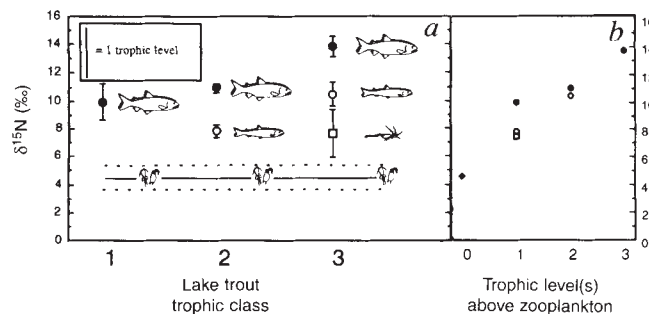


FIG. 2 a, Mean ($\pm$ s.e.) $\delta^{15}\text{N}$ of lake trout dorsal muscle plotted against lake trout trophic class (filled circles). For class 2 and 3 food chains, mean $\delta^{15}\text{N}$ of pelagic forage fish (open circles: average of cisco, smelt, sculpin and/or alewife by lake) and *Mysis relicta* (square) are shown. For fish, sample size per lake was 1–13 (median, 6). Correlation between $\delta^{15}\text{N}$ and class: $r=0.58$, $n=24$, $P<0.01$; significant differences in lake trout $\delta^{15}\text{N}$ ($P<0.05$, Duncan's multiple range test) were found only between either class 1 or class 2 and class 3, but not between class 1 and class 2 lakes. Trout averaged +3.0‰ higher than forage fish in class 2 and class 3 lakes. Addition of *Mysis* to the pelagic food chain (class 3) resulted in the mean $\delta^{15}\text{N}$ of the two next trophic steps (trout and pelagic forage fish) being increased by, respectively, +2.7‰ and +2.6‰ $\delta^{15}\text{N}$ compared to class 2 lakes. In class 2 lakes, pelagic forage fish occupied a trophic position (+3.3 relative to zooplankton) similar to that of *Mysis* in class 3 lakes (+3.0‰ relative to zooplankton). Mean $\delta^{15}\text{N}$ in trout in class 1 lakes was found to be +5.4‰ higher than zooplankton. This last increment in $\delta^{15}\text{N}$ is higher than that expected if trout were fed exclusively on zooplankton (+3.4‰), indicating that trout in some of these lakes feed to a considerable degree on fish, probably from the littoral zone. Feeding studies on a class 1 lake, Lake Louise²⁸, support these large $\delta^{15}\text{N}$ increments, in that lake trout feed actively on littoral fish (cyprinids and catostomids) during winter when they are not thermally isolated from the littoral zone. Average s.e. of duplicate measurements made on a Europa Tracermass mass spectrometer interfaced with a Roboprep-CN analyser was 0.3‰. Lake trout data for class 1 lakes: Anstruther, Happy Isle, Louise (mean $\delta^{15}\text{N}$, $9.9 \pm \text{s.e. } 1.28$); class 2 lakes: Bark, Du Cerf, Opeongo, Papineau, Poisson Blanc, Sherborne, lakes 468*, 442* and 377* (mean $\delta^{15}\text{N}$, 11.0 ± 0.33); class 3 lakes: Bernard, Big Rideau, Clear, Kashagawigamog, Mazinaw, Red Horse, Sand, Ontario, and lake 373* (mean $\delta^{15}\text{N}$, 13.7 ± 0.75). Pelagic forage fish data for class 2 lakes: Linge*, Trout and lakes 468* and 377* (mean $\delta^{15}\text{N}$, 7.83 ± 0.48); class 3 lakes: Kashagawigamog, Mazinaw, Musclow*, Ontario, Orange*, Red Horse and Sydney* (mean $\delta^{15}\text{N}$, 10.38 ± 0.87). *Mysis* data from lakes Ontario and Mazinaw (mean $\delta^{15}\text{N}$, 7.54 ± 1.71). Mean $\delta^{15}\text{N}$ (horizontal solid line) $\pm$ s.e. (dashed lines) for zooplankton (<250 μm) from 31 Ontario and Quebec lakes (mean $\delta^{15}\text{N}$, 4.56 ± 0.50). An alternative indicator of $\delta^{15}\text{N}$ for a primary consumer other than zooplankton was also provided by unionid mussels, which, because of their greater size and longevity and more uniform trophic habits compared with zooplankton, were found to be only a third as variable as zooplankton among lakes (mussel $\delta^{15}\text{N}$ among 9 lakes, s.d. was 0.9; mean, 4.00; zooplankton s.d., 2.78). Asterisks indicate unpublished data from Ontario lakes kindly made available by R. Hesslein (DFO, Canada). b, Mean $\delta^{15}\text{N}$ plotted as a function of the number of trophic level(s) above that of zooplankton. Zooplankton shown as a solid diamond; symbols for the other groups as in a.

Mysis, or of both *Mysis* and pelagic forage fish in, respectively, class 2 and class 1 lakes should be reflected by a corresponding decrease in $\delta^{15}\text{N}$ of lake trout. Indeed, $\delta^{15}\text{N}$ of trout covaried with our three-step food-chain classification, and each of the two intermediate trophic levels, pelagic forage fish and *Mysis*, when present, accounted for increases of nearly 3‰ in $\delta^{15}\text{N}$ of trout (Fig. 2a). Mean trout $\delta^{15}\text{N}$ was positively related to the length of the pelagic food chain, increasing from 9.9‰ in class 1 lakes, through 11.0‰ (class 2 lakes) to 13.7‰ (class 3 lakes). The variable trophic positions of the same organisms in the three food-chain classes were better predicted from $\delta^{15}\text{N}$ values rather than from taxonomy (Fig. 2b).

The comparisons between $\delta^{15}\text{N}$ signature and trophic position made possible by this large dataset, and the ability of the food-chain descriptor to account for the broad variation in nitrogen isotope signature shown by the lake trout and their forage fish, provide a strong test of $\delta^{15}\text{N}$ as a food-web descriptor. By reflecting the effect of continuous among-population variation in food-chain properties such as trophic level and omnivory, $\delta^{15}\text{N}$ will be a more sensitive trophic descriptor and should therefore be a better predictor of contaminant biomagnification than a discrete food-chain classification such as our class variable⁷. Indeed, mercury levels in trout from seven lakes were significantly related to $\delta^{15}\text{N}$ (Fig. 3), but not to trophic class ($r=0.36$, $P>0.4$) (although mercury was significantly related to class in a much larger dataset¹⁹). This suggests that there was considerable variability in the degree of omnivory (feeding at more than one trophic level) within these lake food-chain classes, and that such omnivory influences both the level of contaminant biomagnification and the $\delta^{15}\text{N}$.

Omnivory is an important feature of food webs which has strong consequences, not only for contaminant biomagnification²⁰, but also for energetic efficiencies²¹, top-down feedback^{22–24}, and community stability^{25–27}. The patterns of $\delta^{15}\text{N}$ in our pelagic communities suggest an efficient, time-integrative method of estimating omnivory by comparing the $\delta^{15}\text{N}$ increment observed between two adjacent trophic levels to the increment of 3.4 expected from laboratory studies on pure diets¹.

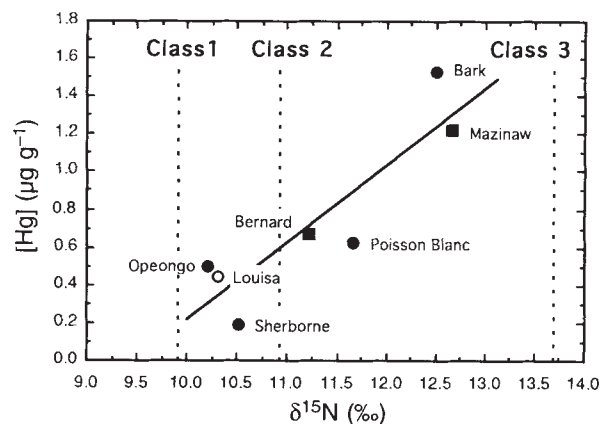


FIG. 3 Relationship between mean mercury concentration (wet weight) and mean $\delta^{15}\text{N}$ in lake trout in seven Ontario and Quebec lakes. Correlation between Hg and $\delta^{15}\text{N}$: $r=0.89$, $P<0.01$. The average $\delta^{15}\text{N}$ for each lake trout trophic class from Fig. 1 are shown as stippled lines. Class 1 lakes are shown as open circles, class 2 as solid circle, and class 3 as solid square. Lakes deviating from their respective class averages in $\delta^{15}\text{N}$ were not outliers on the Hg- $\delta^{15}\text{N}$ plot. For example, Bernard Lake, a class 3 lake which fell well below the class-3 $\delta^{15}\text{N}$ average, had moderate trout mercury levels that were more typical of class 2 lakes. Mercury data are from ref. 19 and were measured in 1976–82, with the exception of Poisson Blanc Lake, for which data were obtained for 1992 from the Quebec government. Samples used for $\delta^{15}\text{N}$ determination were collected in 1991–93.

Although we have not yet obtained sufficiently complete $\delta^{15}\text{N}$ profiles on all of our lakes to calculate the $\delta^{15}\text{N}$ trophic increments on an individual lake basis, the averages that we have calculated for each trophic pair in class 2 and 3 food chains, are all <3.4 , suggesting significant levels of omnivory in these pelagic food webs. To illustrate our approach to estimating omnivory, let us propose that the total ration of the i th trophic (C_i) level can be represented as a weighted sum of the contributions from lower levels

$$C_i = \sum_j \rho_{ij} C_j \quad \text{and} \quad \sum_j \rho_{ij} = 1$$

where the ρ_{ij} are the fractions of the i level's diet made up by each level (j), and for the whole web, $\mathbf{C} = \boldsymbol{\rho}\mathbf{C}$, where $\boldsymbol{\rho}$ is a matrix of the type

$$\begin{bmatrix} 0 & 0 & 0 & 0 \\ \rho_{21} & 0 & 0 & 0 \\ \rho_{31} & \rho_{32} & 0 & 0 \\ 0 & \rho_{42} & \rho_{43} & 0 \end{bmatrix}$$

When the $\rho_{i,i-1} = 1$ and the rest of $\boldsymbol{\rho} = 0$, then the food chain is linear and the $\delta^{15}\text{N}_i$ increments should equal 3.4‰, otherwise there will be omnivory and the $\delta^{15}\text{N}_i$ increments should be <3.4 ‰ because

$$\delta^{15}\text{N}_i = (\rho_{i,i-1}\delta^{15}\text{N}_{i-1} + \rho_{i,i-2}\delta^{15}\text{N}_{i-2} + \dots) + 3.4\text{‰}$$

and for the whole web, $\delta^{15}\text{N} - 3.4 = \boldsymbol{\rho}\delta^{15}\text{N}$.

Although this equation relates the dietary weighting factors (ρ_{ij}) to the $\delta^{15}\text{N}$ profile ($\delta^{15}\text{N}_i$), it only provides explicit solutions for the ρ_{ij} when omnivory (defined as $1 - \rho_{i,i-1}$) is limited to one level, that is $\rho_{i,i-2} = 0$. Otherwise the approach will yield only an envelope, or range of values within which the ρ_{ij} must lie. As dietary studies on class 3 communities in a series of Ontario lakes¹⁷ show that omnivory does in fact only extend to one level (that is, adult lake trout eat *Mysis* in addition to forage fish, but no zooplankton, and pelagic forage fish eat *Mysis* and zooplankton), the ρ_{ij} calculated from our average class-3 $\delta^{15}\text{N}$ values (Fig. 2a), should then be valid. Our estimates based on these $\delta^{15}\text{N}$ values for lake trout indicate only 3% omnivory (97% of their diet is pelagic forage fish), but that omnivory at the next lower level of the food web is substantially higher (19%). Volumetric stomach-content studies on species from six class 3 lakes¹⁷ (different from ours) yield an estimate of 4% (0–15%) omnivory for adult lake trout, and again a much higher omnivory for their forage fish (ciscoes) of 66% (15–90%), paralleling our results based on $\delta^{15}\text{N}$.

Omnivory estimates such as these can be used to model the biomagnification of a persistent contaminant because they allow us to convert models developed under the assumption of linear food chains. Using Thomann's²⁰ steady-state model simplified by the exclusion of the direct uptake term, and written in vector and matrix form, we have $\mathbf{B} = \boldsymbol{\alpha}\mathbf{C}[(\mathbf{K} + \mathbf{G})\mathbf{I}]^{-1}$, where $\mathbf{B}$ is a vector of biomagnification ratios (ratio of tissue contaminant/concentration in prey), and $\boldsymbol{\alpha}$, $\mathbf{C}$, $\mathbf{K}$ and $\mathbf{G}$ represent vectors of assimilation efficiency, ration, contamination excretion rate and growth rate, respectively, for the i elements of the food web, and $\mathbf{I}$ represents the identity matrix.

Such linear food-chain models will overpredict biomagnification in proportion to the degree of omnivory present. Using the omnivory matrix ($\boldsymbol{\rho}$) we can rewrite this model much more realistically as

$$\mathbf{v}(\boldsymbol{\rho}\mathbf{v}\mathbf{I})^{-1} = \boldsymbol{\alpha}\mathbf{C}[(\mathbf{K} + \mathbf{G})\mathbf{I}]^{-1}$$

where $\mathbf{v}$ represents a vector of tissue contaminant levels at the i trophic levels, and $\boldsymbol{\rho}\mathbf{v}$ represents the weighted mean contaminant levels in the diet of each trophic level adjusted for omnivory.

Thus the $\boldsymbol{\rho}$ matrix can be estimated by stable isotope measurements, and these can potentially be used to model contaminant biomagnification much more realistically than is possible using conventional models that assume linear food chains. $\square$

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Evolutionary stability of mutualism between yuccas and yucca moths

Olle Pellmyr & Chad J. Huth

Department of Biology, Vanderbilt University, Nashville, Tennessee 37235, USA

INTERSPECIFIC mutualisms inherently possess a conflict of interests between the interacting species in that fitness increases of one species occur at the expense of the other. This holds for mutualisms as diverse as plant associations with mycorrhizal fungi or nitrogen-fixing bacteria, animals and endosymbionts, and obligate plant-pollinator associations^{1–6}. Prevailing models of interspecific cooperation predict that mutualistic interactions are evolutionarily stable only when both interacting species possess mechanisms to prevent excessive exploitation^{3–6}. In light of this, it is paradoxical that some of the classical examples of coevolved obligate mutualism seemingly do not meet this criterion. In mutualisms involving seed parasites that actively pollinate their hosts, such as yucca/yucca moth and fig/fig wasp interactions, there is no apparent means of retaliation on behalf of the plant. Predictions from theory suggest that a cryptic mechanism, such as selective abortion of flowers with heavy egg loads, could stabilize these interactions^{4,6,9}. Here we present the first empirical evidence that such a mechanism in fact exists in the yucca/yucca moth interaction. A strong negative effect exists between moth egg number and probability of flower retention. Furthermore, we show a strong positive effect between the number of pollinations received and the probability of

flower retention. Selective maturation of fruit with low egg loads and high pollen loads provides a mechanism to increase the quantity and possibly quality of seeds produced, and simultaneously select against moths that lay many eggs per flower or provide low-quality pollinations^{4,6,8,10}. Not only can these results explain the stability of this type of interaction, but selection for high-quality pollination also provides a mechanism to help explain the evolution of active pollination among yucca moths.

The extreme obligate mutualism between yuccas and yucca moths is one of the most frequently cited cases of interspecific cooperation^{6,11–13}. Shortly after emergence, the female moth uses specialized mouthparts to collect pollen from yucca flowers and carries a pollen load for most of her brief life. Oviposition takes place inside flowers, where she cuts into the ovary with a piercing ovipositor and attempts to deposit an egg. She then walks up to the stigma, and actively deposits a small amount of pollen. In so doing, she ensures that lack of pollen will not limit the availability of developing seeds, which is the exclusive food of her progeny. Oviposition may be repeated several times within a flower before she moves on. Upon hatching, the larva consumes only a small proportion of the developing seeds, leaving

many intact^{11,13,14}. Reciprocal plant specialization through exclusion of other pollinators has led to obligate mutual dependence between yuccas and their pollinators^{15,16} as neither species can successfully reproduce without the other.

The nature of this interaction results in an evolutionary conflict between the moth and its host which may lead to breakdown of the system. Moths can attain higher relative fitness by increasing egg loads within individual flowers but this would directly reduce yucca fitness in terms of intact seeds. Evolutionary stability of the interaction requires that the plant possess a mechanism allowing it to prevent heavy larval damage^{3,4,6}. Here we present the first empirical evidence for the existence of the long-predicted mechanism of selective abortion.

We used *Yucca filamentosa* (Agavaceae) and its exclusive pollinator, *Tegeticula yuccasella* (Lepidoptera: Prodoxidae), to test for the presence of moth-induced selective abortion. A native of the eastern North American seaboard, *Y. filamentosa* was naturalized across the eastern United States during the 1800s and the moth rapidly colonized its host throughout the extended range^{17,18}. Our experiments were done at the Spring Grove Arboretum in Cincinnati, Ohio, where a large population (>350 flowering stalks per year) has been established for 110–120 years and pollinators for >90 years (O.P., unpublished data). Inflorescences have 165–475 flowers which open in overlapping sequence over 10–20 days. Each flower is open for 1–2 days.

The selective-abortion hypothesis predicts that, whenever resources limit fruit set, flowers with the heaviest egg loads should be selectively aborted by the plant. Resource limitation was tested by comparison of fruit set among 20 inflorescences, all of which were naturally pollinated while half of them also received supplemental pollen to all flowers. Fruit set was low ($\bar{x} = 13.4 \pm 6.1\%$), as is characteristic of yuccas^{13,19,20}, but not significantly different between treatments. Thus, fruit set was resource-limited and the criterion for expecting selective abortion was met.

Moth visitation of flowers was determined through dissections of all retained and aborted flowers from ten naturally-pollinated inflorescences, scoring each flower or fruit for number of moth eggs and oviposition scars. We measured both parameters because females often fail to deposit an egg: 35.7% of all recorded attempts ($n = 7,927$) failed. The moths do, however, pollinate flowers as often after a failed attempt as after a successful oviposition; data from 65 moth behaviour sequences on virgin flowers showed that the total number of oviposition attempts was a stronger predictor than egg number of the number of pollinations (attempts: $R^2 = 0.563$, $P < 0.001$; eggs: $R^2 = 0.336$, $P < 0.001$; homogeneity among correlation coefficients: $\chi^2 = 3.032$, $P < 0.086$). Thirty-eight per cent of all flowers ($n = 2,085$)

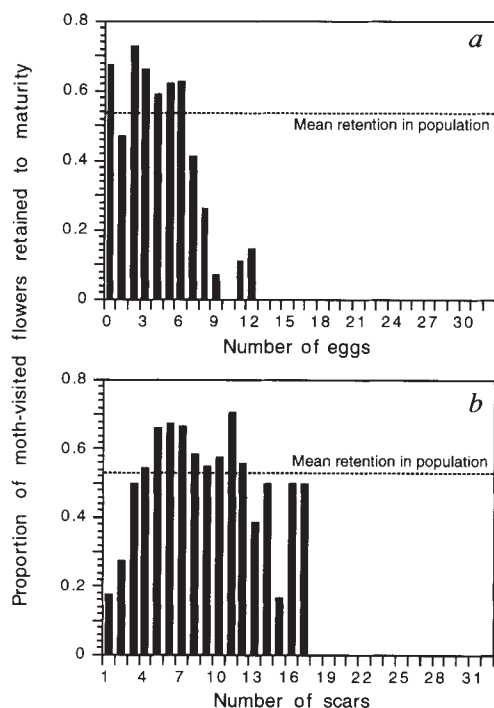


FIG. 1 Proportion of retained flowers for each *Tegeticula* egg and scar number category. Dashed lines indicate grand mean retention in the entire sample. Flowers were tagged on their opening day with a $1 \times 3 \text{ mm}^2$ label giving plant identity and date. Abscised flowers were collected each morning throughout the flowering period and fruit maturation, and ripe fruits were harvested just before dehiscence. During dissection, the number of moth eggs (exit holes for ripe fruit) and total number of oviposition scars were recorded. 10.5% of the labelled flowers were excluded because degradation was too extensive to allow safe counts. For the present analysis, all flowers attacked by the nitidulid beetle *Carpophilus melanopterus* were removed. Larvae and adults of this beetle attack all stages from buds to young fruit and invariably cause abscission. Thus the cause of abortion could not be determined in these flowers. A total of 559 moth-visited flowers were used in the analyses. Flowers with high egg and scar numbers were pooled to meet sample-size requirements for statistical analysis: in a, 15–17 eggs, 18–20 and >20 eggs were combined as cohorts; in b, 10–11 scars, 12–14, 15–17 and >17 scars were combined. Sample sizes by category for the eggs (0–28) were: 49, 64, 85, 68, 68, 58, 48, 39, 23, 14, 8, 9, 7, 3, 5, 1, 4, 1, 2, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, and for the scars (1–32): 40, 29, 32, 59, 68, 64, 60, 55, 40, 26, 27, 18, 13, 6, 6, 2, 4, 4, 1, 2, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1.

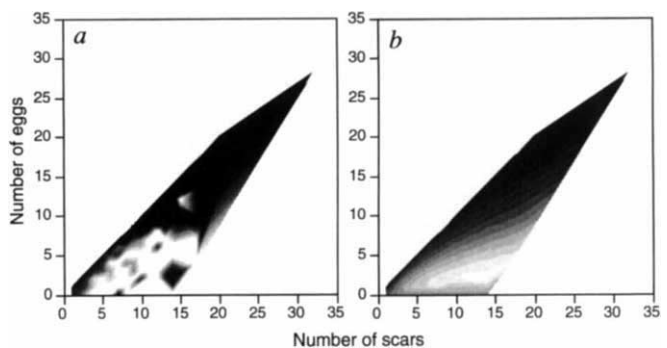


FIG. 2 Relative probability of flower retention for different combinations of egg and scar numbers, based on a, actual frequencies in the sample data; and b, the best-fit model for predicted retention probabilities derived in the multivariate analysis (Table 1). Lighter shades indicate higher probability of retention. Note that egg number cannot exceed scar number. Unplotted egg and scar coordinates were outside the range of observed occurrences.

TABLE 1 Multiple regression predicting probability of retention of undamaged, pollinated yucca flowers

Variable	Partial regression coefficient	Standard error	Standardized coefficient	T	P(2-tailed)
Constant	1.474	0.061	0.0	24.325	<0.001
Egg	-0.028	0.006	-0.201	-4.525	<0.001
$e^{-\text{egg}}$	-0.737	0.103	-0.420	-7.123	<0.001
$e^{-\text{scar}}$	-0.521	0.246	-0.101	-2.116	0.035
Egg/scar	-1.083	0.088	-0.692	-12.297	<0.001

Analysis of variance					
Source	Sum of squares	Degrees of freedom	Mean square	F-ratio	P
Regression	57.220	4	14.305	96.997	<0.001
Residual	81.703	554	0.147		

Multiple regression was done on ten naturally pollinated inflorescences ($n=559$ flowers) using forced entry of the independent variables into the regression.

on the experimental inflorescences received moth oviposition attempts. Scar numbers ranged between 1 and 32, and egg numbers from 0 to 28. Figure 1 shows the proportion of fruit matured for moth-visited flowers based on number of eggs and oviposition attempts. The probability of flower abortion was significantly non-random ($\chi^2=89.182$, $P<0.001$), with flowers carrying heavy egg loads being aborted disproportionately often (Fig. 1a) and thus strongly supporting the selective-abortion hypothesis.

The least egg-laden flowers, however, did not have the highest retention probability as predicted by the selective-abortion hypothesis (Fig. 1a). It is possible that the primary cost to the plant, seed consumption, is not the only factor determining moth-induced abortion. The major cooperative behaviour of the moth, pollination, may also affect fruit maturation, as the amount of pollen deposited on stigmas can affect progeny vigour^{21,22}. Differential fruit maturation based on pollen quantity¹⁰ has never been incorporated in selective-abortion hypotheses for yuccas, but it would allow the plant to adjust the quality of its progeny within the constraint of limiting resources. Because oviposition attempts are positively correlated with pollination events, we used them as an indicator of pollen amount. Retention probability was strongly dependent on the number of oviposition scars ($\chi^2=60.767$, $P<0.001$), with flowers having 4 to 12 scars being preferentially retained (Fig. 1b). A consequence of few oviposition attempts, although theoretically beneficial in terms of cost for the plant, is reduced retention probability of the least egg-laden flowers, which is evidently due to insufficient pollination.

Our results demonstrate that oviposition and pollination behaviour have opposing effects on the probability of fruit reten-

tion, causing preferential retention of flowers with lower egg numbers and higher scar numbers (Fig. 2a). Retention probability can be predicted from multivariate analysis using regressions based on observed egg load, scar number and corresponding interaction terms. Several different multiple regressions were calculated using forced-fit procedures^{23,24}. The best model of relative retention (Table 1; $R^2=0.412$, $P<0.001$) included linear and exponential terms of egg and scar numbers and the egg/scar ratio; all factors had highly significant partial regression coefficients. Graphic representation of the estimates in the retention probability model (Fig. 2b) show a strong selective trade-off in the yucca flowers, favouring high pollen load but low egg numbers. This trade-off can effectively stabilize the mutualism in that these mechanisms increase plant progeny quantity and possibly quality while selecting against moths that exploit the system by laying a large number of eggs or providing low-quality pollination. The assumption that plant fitness is increased by selective abortion is based on phylogenetically widespread positive correlations between pollination levels and progeny fitness^{10,22}; long-term data on fitness differentials in *Y. filamentosa* based on pollen quantity and genotype could provide for estimation of optimal abortion patterns in the plant.

The proximal mechanism of abortion are not yet known, but the temporal pattern (Fig. 3) allows exclusion of two common causes of abortion. Virtually all abortion occurred before the moth eggs hatched, excluding larval feeding as a factor, and oviposition scar numbers were negatively correlated with abortion so external damage is counter-indicated as well. It is not known whether the mechanisms represent coevolved traits or preadaptations in the yuccas, but this does not affect their significance in contributing to maintaining a mutualistic equilibrium. It will be important to explore, however, for understanding the role of coevolution in highly specialized interactions.

The linkage between pollen amount and fruit retention in yuccas provides a powerful mechanism to explain the evolution and maintenance of the highly unusual trait of active pollination. Because all extant yuccas are pollinated by moths, and yuccas represent some of the most derived members of the Agavaceae²⁵, traits of the ancestor first colonized by a non-pollinating moth must be inferred from character states in more basal genera. Pertinent traits thus inferred include strong resource limitation of fruit set²⁶, nectar production²⁷, ineffective pollination by a broad spectrum of nectarivores^{26,28} and possibly selective abortion²⁹. The colonizing moth ancestor thus faced very substantial loss of progeny to floral abortion^{20,30}, and any factor reducing abortion probability of a particular fruit where a female has laid eggs would be under strong positive selection. Although the ancestral pollinators may have provided sufficient pollination to maximize fruit set, as is the case in extant agaves²⁶, moths would still be under strong selection to provide high-quality pollination as it directly affects retention probability of a given fruit. This competition-dependent mechanism is sufficient to maintain selection for increasingly effective pollination among

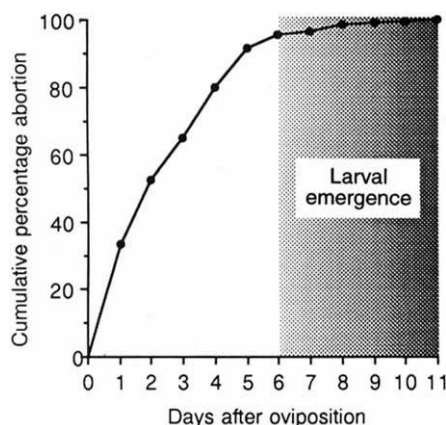


FIG. 3 Temporal pattern of abortion of all aborted flowers in Fig. 1. Egg-hatching dates were determined by scoring whether any moth larvae were present in each dissected flower. Note that >95% of all undamaged flowers had aborted by the time that larval feeding commenced.

the yucca moths. Importantly, the evolution of obligate pollination behaviour could evolve in the moths during a period of coexistence with less effective ancestral pollinators and without any counteradaptation in the host. □

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Word recognition in the human inferior temporal lobe

Anna C. Nobre*, Truett Allison & Gregory McCarthy†

Neuropsychology Laboratory, Veterans Administration Medical Center, West Haven, Connecticut 06516, USA, and Section of Neurosurgery and Department of Neurology, Yale University School of Medicine, New Haven, Connecticut 06510, USA

STUDIES of primates¹ and of patients with brain lesions² have shown that the visual system represents the external world in regions and pathways specialized to compute visual features and attributes. For example, object recognition is performed by a ventral pathway located in the inferior portion of the temporal lobe³. We studied visual processing of words and word-like stimuli (letter-strings) by recording field potentials directly from the human inferior temporal lobe. Our results showed that two discrete portions of the fusiform gyrus responded preferentially to letter-strings. A region of the posterior fusiform gyrus responded equally to words and non-words, and was unaffected by the semantic context in which words were presented. In contrast, a region of the anterior fusiform gyrus was sensitive to these stimulus dimensions. These regions were distinct from areas that responded to other types of complex visual stimuli, including faces and coloured patterns, and thus form a functionally specialized stream within the ventral visual pathway.

Within the ventral visual pathway, regions specialized for processing faces^{4,7} and colours⁸ have been identified in monkeys. In humans, imaging studies using positron emission tomography (PET)^{9,12} and intracranial recordings of event-related potentials (ERPs)^{13,14} have localized these functional areas in regions of extrastriate cortex. Studies of brain lesions^{15,17} and cortical stimulation¹⁸ in patients suggest that there is a specialized region for the processing of written words in the inferior temporal lobe. One PET study¹⁹, but not another²⁰, reported increased blood flow in the inferior temporal lobe during reading. We investigated the role of the inferior temporal lobe in word recognition by recording cortical surface ERPs directly from the inferior temporal and occipital lobes in 27 patients (381 electrode locations overall) while they performed tasks involving visually presented letter-strings, faces and control stimuli (Fig. 1).

Two types of ERPs elicited by letter-strings were recorded in the fusiform gyrus. Large negative field potentials with peak latencies near 200 ms (N200) were elicited in discrete regions of

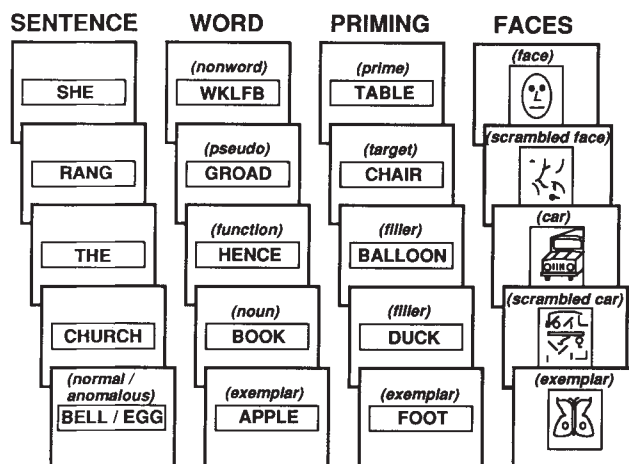


FIG. 1 Behavioural tasks. Twenty-seven patients (13 women, 23 right-handed, 18–55 years of age) with medically intractable epilepsy, who were being evaluated for possible neurosurgery²⁷, viewed a computer monitor placed 60 cm from the eyes. Language lateralization was tested using the sodium amyltal procedure in 22 patients. All were left-hemisphere dominant. In the sentence task²⁸, words in each of 160 sentences were presented briefly (100 ms duration) and rapidly (500–800 ms stimulus-onset asynchrony, SOA). Half the sentences terminated with a semantically correct best completion, thus the normal terminal word was primed by sentence context. Words or letter-strings subtended visual angles of 3–10° horizontally and 0.5–1.0° vertically. Patients read the sentences silently for comprehension and were told that some endings might appear odd. Debriefing ensured that patients read the sentences. In the word and priming tasks²², 220 stimuli were presented (500 ms duration, 2.0–2.1 s SOA). In the word task, patients detected names of fruits and vegetables (10% of the stimuli). The remaining letter-strings were equally divided among non-pronounceable non-words, pronounceable non-words (pseudowords), words serving grammatical function, and concrete nouns. In the priming task, patients detected names of body parts (10% of the stimuli). The remaining words were all concrete nouns. Half were pairs of semantically related nouns, with the first word of each pair designated the prime and the second word the target. The remaining half were unrelated word pairs (fillers). The face task required categorization of stimuli (250 ms duration, 1.8–2.0 s SOA) subtending 7.2 × 7.2° of visual angle¹⁴. Patients detected pictures of butterflies (10% of the stimuli). The remaining stimuli were equally divided among faces, digitally scrambled faces (equiluminant with the original stimulus but unrecognizable as faces), cars and scrambled cars. Other control stimuli included black-white or coloured checkerboards. The requirement to make key presses to exemplars ensured the patient's attention to the stimuli and provided another measure of ERP selectivity to category-specific stimuli.

* Present address: Department of Experimental Psychology, Oxford University, South Parks Road, Oxford OX1 3UD, UK.

† To whom correspondence should be addressed.

the posterior fusiform gyrus by all types of letter-strings but not by other stimuli (Fig. 2). Letter-string type (locations 1 and 2) and semantic priming (locations 3 and 4) did not affect N200. The exact stimulus attributes necessary to generate letter-string N200s are unknown, but at most locations they are not generated by faces (location 1), complex biological (location 2) or non-biological (location 4) objects and by checkerboards (location 3).

The second type of ERP elicited by letter-strings was recorded in the anterior portion of the fusiform gyrus (Fig. 3). This ERP was a broad positive potential with a peak near 400 ms (P400).

In marked contrast to the posterior N200, the anterior P400 was sensitive to properties of letter-strings. Words with semantic content elicited larger P400s than words with grammatical function (location 2). Semantic priming by pre-exposure to a related word (locations 3 and 4) or by sentence context (locations 1 and 3) diminished or abolished P400. P400 was not elicited by non-words (location 4) or by a variety of control stimuli including checkerboards (location 1) and faces (location 2). Electrodes placed inside the anterior temporal lobe near the cortical surface recorded a broad negative ERP with peak latency and waveshape equivalent to P400 (refs 21, 22). Such polarity inversions²³ indicate that P400 is locally generated in the anterior fusiform gyrus.

The anatomical and functional specificity of N200 and P400 is illustrated for a single patient in Fig. 4a. A letter-string N200 (location 1, solid arrow) was recorded medially and posteriorly to a face N200 (ref. 14; location 3, curved solid arrow); this spatial differentiation was seen in six of eight patients in whom

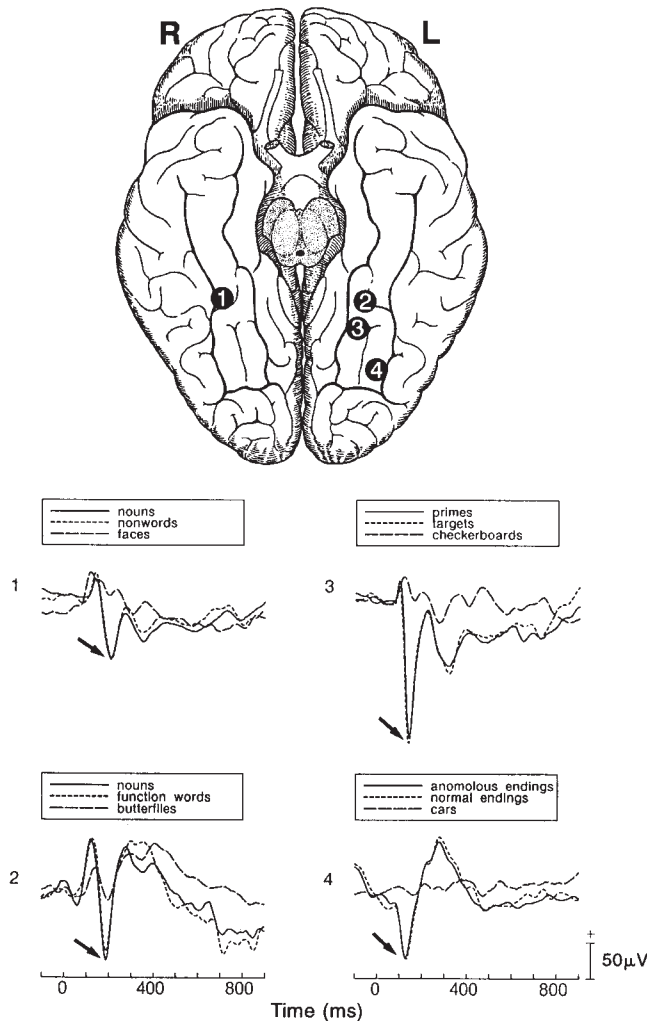


FIG. 2 Examples of letter-string N200s (arrows) in posterior fusiform gyrus. Strips of 8–12 electrodes (2.2 mm diameter, 1.0 cm interelectrode distance) were placed on the cortical surface of the brain for 3–21 days. The electroencephalogram was recorded simultaneously from 32–64 electrodes referential to a mastoid (250 Hz digitization rate; 10,000 gain; 0.1–100 Hz band pass). EEG segments were segregated by stimulus type and averaged. Electrodes were localized by post implant magnetic resonance images. Electrode locations are depicted using gyral anatomy in the lateral axis. Coordinates of Talairach and Tournoux²⁹ were used for the anterior–posterior axis. ERPs from four patients are depicted (locations 1–4). In each example, large N200s were elicited by letter-strings (nouns, non-words, function words, primes, targets, normal and anomalous sentence endings) but not by control stimuli (faces, butterflies, high-contrast checkerboard patterns, and cars). Within patients, the amplitudes and latencies of the letter-string N200s did not vary with the properties of the letter-strings. Waveforms on location 3 are from a left-handed patient. All other data in this and subsequent figures are from right-handed patients.

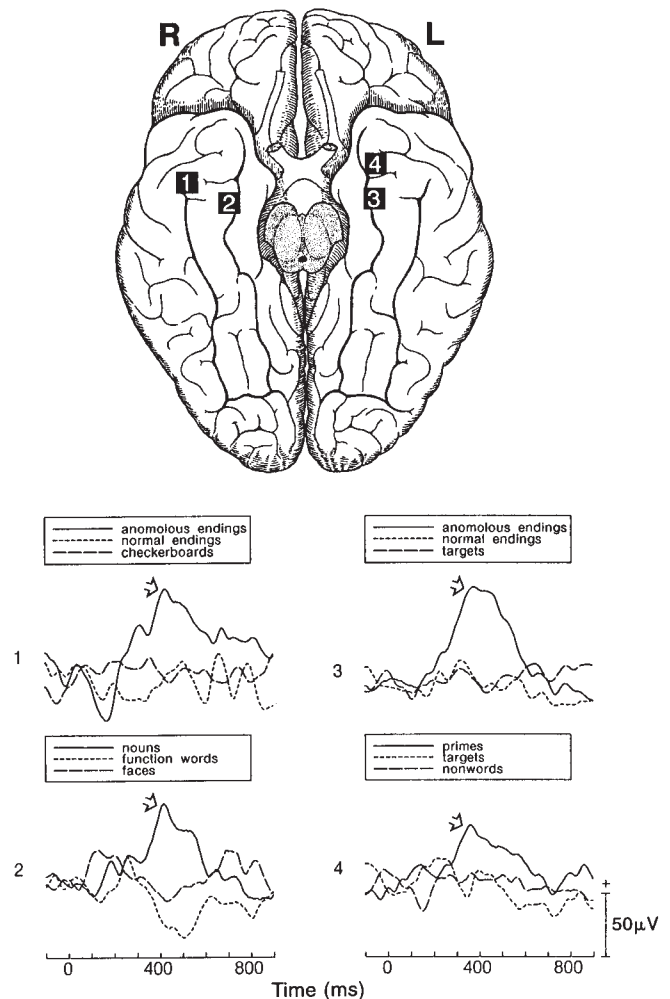


FIG. 3 Examples of letter-string P400s (arrows) in anterior fusiform gyrus. ERPs from four patients are depicted (locations 1–4). In each example, large P400s were elicited by nouns (anomalous sentence endings, locations 1 and 3; and unprimed nouns, locations 2 and 4) but not by control stimuli (checkerboards, location 1; faces, location 2). P400 was sensitive to the properties of letter-strings and to the context in which nouns were presented. Semantic priming (targets, locations 3 and 4) or priming by sentence context (normal sentence endings, locations 1 and 3) diminished or abolished P400. Function words (location 2) and non-pronounceable non-words (location 4) did not elicit P400.

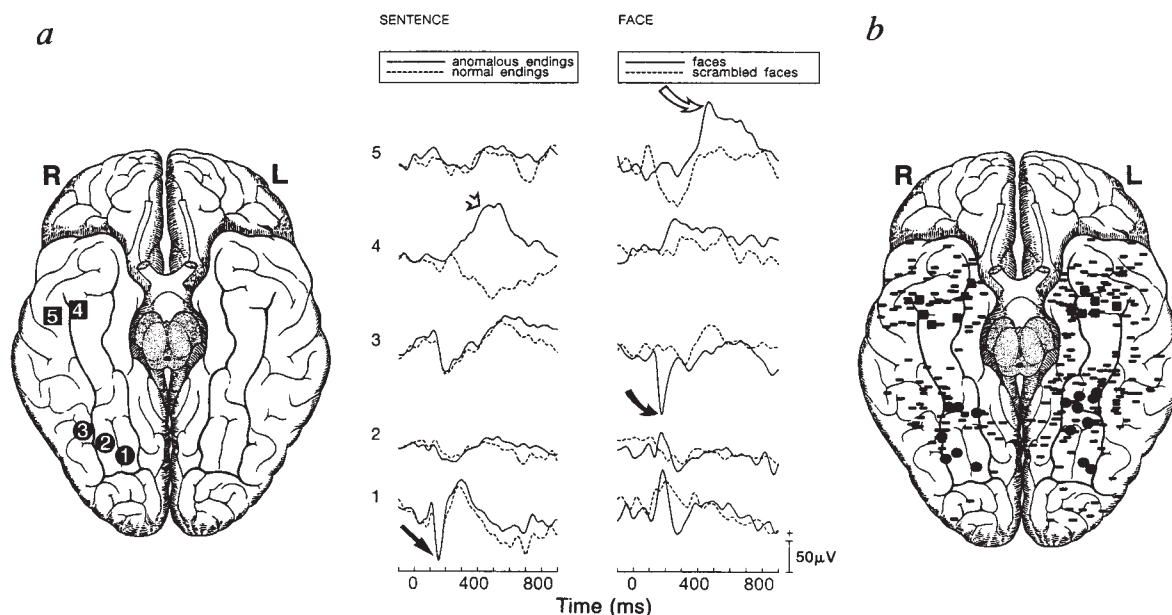


FIG. 4 Modularity and modulation of letter-string ERPs. *a*, Recordings from the sentence task are compared with recordings from the face task in a patient. A letter-string N200 (arrow) was observed at electrode location 1 in the posterior fusiform gyrus. In contrast, location 3 of the same electrode strip recorded a face N200 (curved arrow) in the inferior temporal gyrus. A letter-string P400 (open arrow) was elicited at location 4 in the anterior fusiform gyrus, while a face P400 (curved open arrow) was elicited at location 5 in the anterior inferior temporal gyrus. The letter-string N200 was not sensitive to priming in sentences, whereas

the letter-string P400 was markedly reduced. As in previous reports^{14,24}, the face N200 and P400 were specific to faces. The sensitivity of the face P400 to context is at present unknown. *b*, Electrode locations of letter-string N200 (●), letter-string P400s (■), and locations where letter-strings did not elicit selective ERPs (□) are shown for all patients. Mean Talairach locations²⁹ were: N200 left, X=G.18, Y=lc.05; N200 right, X=H.03, Y=rc.00; P400 left, X=E.21, Y=lb.46; P400 right, X=E.43, Y=rc.17.

N200s were recorded both to letter-strings and faces. Similarly, P400s elicited by words (open arrow) and faces²⁴ (curved open arrow) were recorded from separate locations (4 and 5) in the anterior temporal lobe. The locations from which all letter-string N200s and P400s were recorded are summarized in Fig. 4*b*. Both letter-string and colour recordings¹³ were obtained in five patients; in all cases N200s were generated by letter-strings at locations lateral to those that generated the largest colour ERPs.

These results suggest that there is a separate stream specialized for word recognition within the ventral visual pathway, and that a sequence of processing occurs over a period of ~200 ms. Two putative nodes of the word stream appear to compute increasingly higher-order characteristics of words. In discrete regions of the posterior fusiform gyrus, N200s are elicited by all types of letter-strings, and may reflect the formation of a word percept. Important stimulus features for processing in the word-percept node may be the characteristic configuration of small elements arranged in a linear array, the requirement for integrating multiple elements to yield an object¹⁷, or the grouping of letters into recognizable word forms²⁵. Given their insensitivity to word type, letter-string N200s probably reflect pre-lexical processing of words or possible words. This pre-lexical node is posterolateral to a possible lexical region of the left inferior medial temporal lobe activated in PET studies by real words and pronounceable non-words¹⁹.

In discrete regions of the anterior fusiform gyrus, P400s are sensitive to semantic content and context, and thus may correlate with the activation of a word concept. The word-concept node may form visual or multimodal image-based representations of words or may serve a mnemonic function in associating a stimulus with its context, emotional valence or verbal label²². A mnemonic role is consistent with the proximity of the anterior fusiform region to entorhinal cortex, hippocampus and amygdala. The fusiform gyrus is one of several structures often damaged in alexic and agnosic patients¹⁵⁻¹⁷. Damage to the word-

recognition stream in the fusiform gyrus may help to explain some forms of reading and object-naming deficits in patients with brain lesions^{2,15-17}, such as the posterior or occipital form of alexia²⁶.

Across patients, the amplitudes and latencies of N200 and P400 were similar in both hemispheres, suggesting that the word processing described here is bilateral. However, electrodes were rarely placed symmetrically in the two hemispheres, making it difficult to assess laterality. Additional nodes in language-specific regions of the dominant superior temporal lobe may be interposed between the word-percept and word-concept nodes. The proximity of these nodes in the fusiform gyrus suggests the intriguing possibility that semantic or conceptual representations of words may also be accessed directly within the ventral pathway. □

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A self-renewing multipotential stem cell in embryonic rat cerebral cortex

Andrew A. Davis* & Sally Temple†

† Division of Neurosurgery and *† Department of Pharmacology, Albany Medical College, Albany, New York 12208, USA

NEUROECTODERM cells in the cortical ventricular zone generate many diverse cell types, maintain the ventricular zone during embryonic life and create another germinal layer, the subventricular zone, which persists into adulthood^{1,2}. In other vertebrate tissues, including skin, intestine, blood and neural crest, stem cells are important in maintaining a germinal population and generating differentiated progeny^{3–6}. By following the fates of single ventricular zone cells in culture, we show here that self-renewing, multipotential stem cells are present in the embryonic rat cerebral cortex. Forty per cent of these stem cells produced all three principal cell types of the central nervous system: neurons, astrocytes and oligodendrocytes. Stem cells constituted about 7% of cortical clones; in contrast, over 80% consisted of small numbers of neurons or glia. We suggest that multipotential stem cells may be the ancestors of other cortical progenitor cells that exhibit more limited proliferation and more restricted repertoires of progeny fates.

We analysed the development of 464 clones (Table 1) derived from embryonic day-12 (E12) and E14 cerebral cortices, which consist almost entirely of ventricular zone cells^{1,2}. The majority (~80%) were small clones of neurons, defined by morphology

and staining for neurofilament protein (Fig. 1a, b and Table 1). About 2% of single cells gave solely astrocytic or solely oligodendrocytic clones, judged by staining with antibodies against glial fibrillary acidic protein, which labels astrocytes (Fig. 1c and d) or with antibodies O4 and O1, which label oligodendrocytes⁷ (Table 1). In contrast, about 7% of single cells continued to divide for weeks and produced hundreds of progeny. We examined the differentiated cell types present in these large clones and found that ~40% contained neurons, astrocytes and oligodendrocytes, revealing a common precursor for the three major classes of cells from the central nervous system (CNS) (Fig. 2). The remainder appeared to contain only neurons and astrocytes or only astrocytes and oligodendrocytes.

We investigated whether the cortical cells that generated large, multiple-type clones were stem cells. A defining characteristic of stem cells is their ability to renew themselves^{3,4,8}. To test for self-renewal, we grew putative stem cell clones until they contained 20–30 cells (~4 days of culture), dissociated them and subcloned individual progeny into culture wells. If the initial cell had undergone self-renewal, some of the progeny would behave as stem cells, generating large, diverse clones. We found that this was the case in all eight clones that were subcloned. For example, for one 26-cell clone, ten subcloned progeny survived, seven generated clones of 1–5 cells, one gave 16 astrocytes, and two generated hundreds of progeny of multiple cell types (Fig. 3). This indicates that the initial cell was probably a stem cell capable of self-renewal.

The frequency of progenitor cell types was similar at E12 and E14; 76% of E12 and 81% of E14 cortical ventricular zone cells differentiated into one or two neurons. The frequency of stem cells at E12 and E14 was $7.4 \pm 7.5\%$ versus $7.3 \pm 1.5\%$ respec-

FIG. 1 Three of the clonal types. a, Phase micrograph of a single neuron, the most common product of the isolated ventricular zone cells. b, Larger neuron-only clone consisting of four progeny. c, Phase micrograph of a clone consisting solely of astrocytes. d, Astrocyte clone evident after staining with antiserum against GFAP, visualized with a fluorescein-labelled anti-rabbit antibody (Tago). Scale bar, 100 μ m. Single E12–E14 cortical cells were mechanically dissociated in hibernation medium²⁷ and plated as one cell per Terasaki well by micromanipulation¹⁹. Culture medium was a serum-free medium (DMEM B27 plus N2 (Gibco)) which had been conditioned by cortical astrocytes and meningeal cells²⁸. Culture wells contained sonicated membrane homogenates from C6 cells at 0.01 mg protein per ml. The C6 membrane supports division of isolated embryonic cortical ventricular zone cells²⁹. Under these culture conditions about 85% of single E12–E14 cortical cells survived the first 24 h of culture, as judged by the presence of a live cell or its progeny in the culture wells.

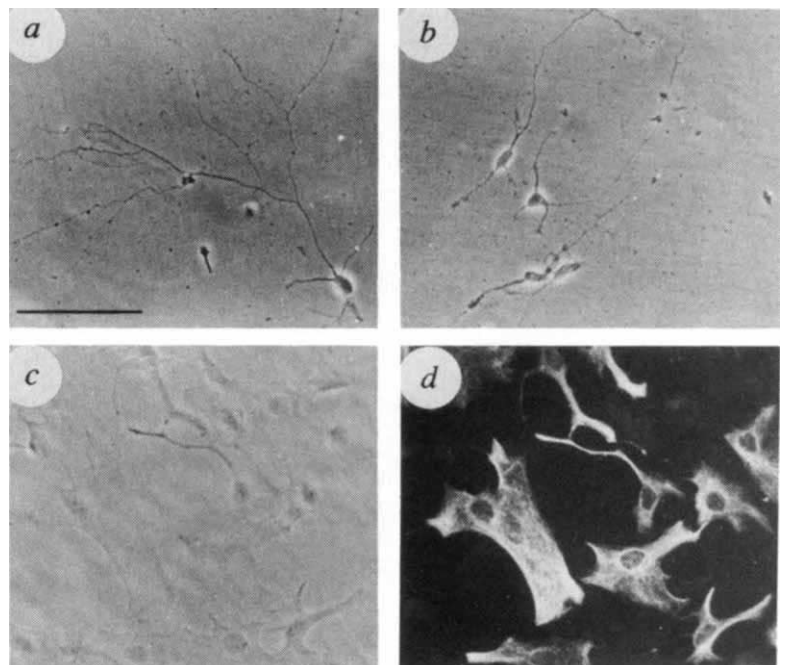


TABLE 1 Frequency of clonal types from single cells cultured from E12 and E14 cerebral cortex

Age		Clone composition				Mixed neurons and non-neuronal* (<100 cells)	Stem cells†	Total clones
		Single neuron	Pairs of neurons	>2 neurons	Astrocytes or oligodendrocytes			
E12	Total of 3 replications	114	48	9	3	22	27	223
	As a percentage of 3 replications	50 ± 4.3%	26 ± 6.9%	6.4 ± 4%	1.9 ± 0.8%	9.2 ± 1.6%	7.4 ± 7.5%	
E14	Total of 3 replications	143	53	8	4	17	16	241
	As a percentage of 3 replications	59.6 ± 14.2%	21 ± 8.8%	2.6 ± 3%	1.3 ± 0.6%	8 ± 4.6%	7.3 ± 1.2%	

See legend to Fig. 1 for conditions. Cells were classified using morphological criteria and cell-type-specific markers. Markers included antibodies O1 and O4 (Boehringer–Mannheim), both of which stain the surface of oligodendrocytes⁷, antibody RT97 (Developmental Studies Hybridoma Bank) against neurofilament protein for neurons, and an antiserum to glial fibrillary acidic protein (GFAP) (Dako) for astrocytes.

* Average size of mixed clones was 25 for E14 and 29 for E12 cortex.

† Stem cell clones consisted of hundreds of cells of different neural phenotypes.

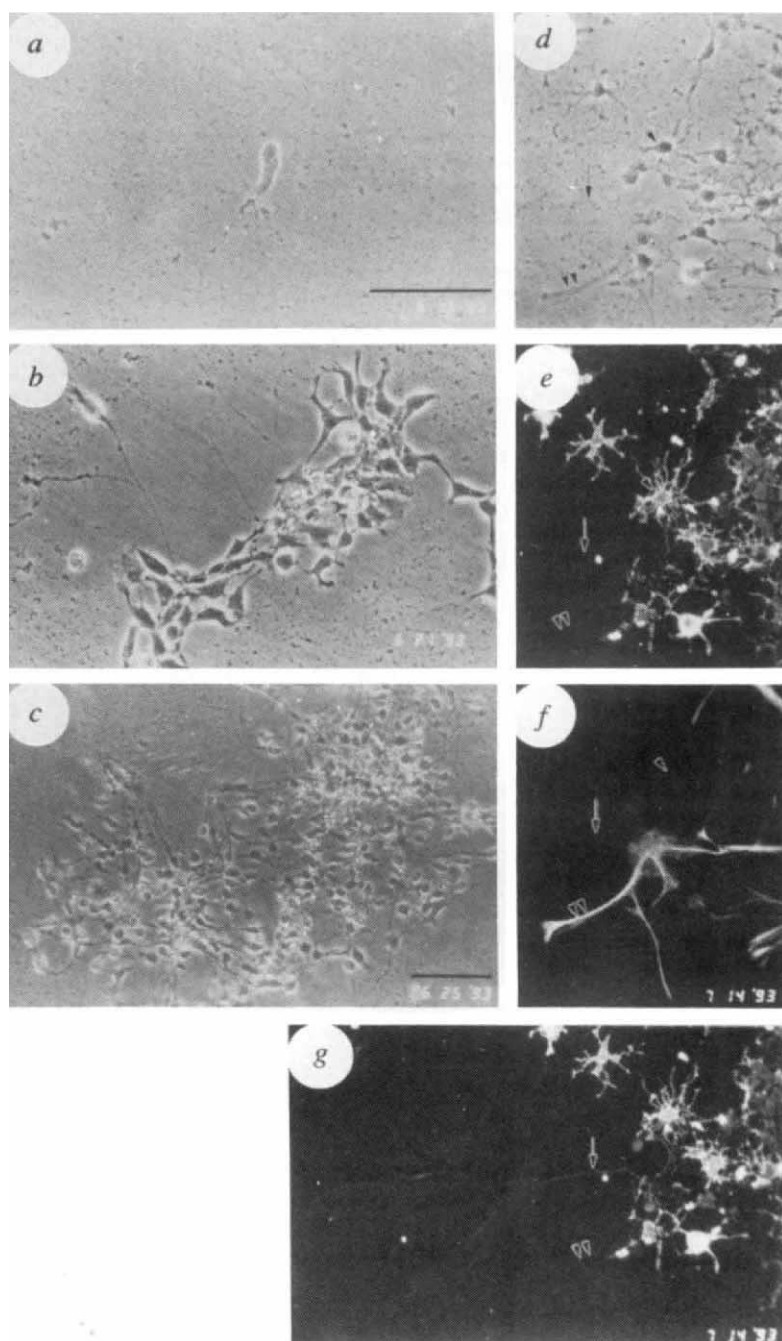


FIG. 2 A multipotent stem cell that generated neurons, astrocytes and oligodendrocytes. *a*, Two progeny of the original single cell after 1 day of culture; *b*, same clone after 6 days of culture. Note the increase in cell number and the differentiation of neuron-like cells. *c*, Further growth and differentiation of the clone by 10 days *in vitro*. *d*, Phase micrograph of a section of the culture at 20 days showing the morphology of cells subsequently stained with antibody markers to CNS cell types. The cell markers were used sequentially and never overlapped in a single cell. *e*, The clone after staining with O4 monoclonal antibody, visualized with a rhodamine-conjugated anti-mouse antibody (Tago). Cells with the morphology of oligodendrocytes are clearly labelled with O4. *f*, Clone after fixation, permeabilization and staining with an antiserum to GFAP, visualized with a fluorescein-conjugated, anti-rabbit antibody. GFAP-positive astrocytes are labelled. *g*, Clone after staining with a monoclonal antibody to neurofilament protein (RT97, visualized with a rhodamine-conjugated anti-mouse antibody). Fine processes of neurons are labelled; these are shown (arrows) to be present in the phase micrograph (*d*), to be negative for O4 (*e*) and GFAP (*f*). Note that in *g* the O4-labelled cells are also visible with rhodamine labelling from the first round of cell-surface staining. Scale bar, 100 μ m.

tively (Table 1). After six days of culture, E12 stem cell clones were significantly larger (mean, 65.2 cells) than E14 stem cell clones (mean, 33.3 cells) ($P < 0.05$). The underlying reason for the faster growth rate of E12 stem cells, possibly decreased cell-cycle time, reduced cell differentiation or reduced cell death, remains to be determined.

We compared clones in single-cell culture to those reported using retroviral vector labelling of murine cerebral cortex at a similar developmental age and found a close correlation between the composition of neuronal clones. For example, the average size of multicellular neuronal clones was 3.23 ($n = 57$) for E12

versus 2.36 for E14. Retroviral labelling of E12–E14 mouse cerebral cortex yielded 83% purely neuronal clones, averaging 3.5 cells for injections made at E15 and 2.0 cells at E17 (ref. 10). But in the case of glial clones, we found a much lower percentage (2%) of purely astrocytic or oligodendroglial clones than reported from retroviral labelling studies at similar ages *in vivo* (12–40%)^{9,12}. There may be selection against the development of immature glia in our culture system, although some can divide and differentiate. Alternatively this difference could be explained if some glial progeny of ventricular zone cells *in vivo* migrated through the developing cortex, creating additional subclones, as suggested from patterns of retrovirally labelled glial clones *in vivo*.^{13,14}

The most notable conflict between the two sets of data is the fact that a multipotential stem cell has not been found by retroviral lineage tracing *in vivo*. Although occasional retrovirally labelled clones containing neurons and glia have been seen *in vivo*^{9,10,11,15}, they are significantly smaller than stem cell clones *in vitro*. Perhaps ventricular zone cells acquire stem cell properties and generate large clones under these culture conditions (which include glial membranes and conditioned medium) although they are constrained to yield small clones *in vivo*. Alternatively, stem cells may not have been scored *in vivo*: the occasional observation of a large, multicell-type, widespread (due to cell migration^{10,16}) clone might have been difficult to define as the product of a single infection^{9,16}.

Because most retrovirally labelled clones *in vivo* consist of one cell type^{9,10,11}, even of one neuronal subtype¹², it has been suggested that most cortical progenitor cells are restricted in their fates^{12,14}. We also observed mainly single-type clones, even in conditions supporting a multipotential fate, and in over 95% of neuron-only clones, all the progeny appeared to differentiate before cell death occurred. Our data therefore support (but do not prove) the idea that these progenitor cells were restricted.

The coexistence of a minor stem cell population among a larger population of apparently more restricted progenitor cells is reminiscent of the blood system⁵. By analogy with haemopoiesis, we propose that multipotential stem cells form the foundation of the cerebral cortex: self-renewing to produce and maintain cortical germinal layers, and dividing asymmetrically to generate different restricted progenitor cells that amplify individual cortical cell types, probably through symmetric divisions. As neurogenesis precedes gliogenesis *in vivo*, we suggest that the restricted progenitor cells generated early in development are predominantly neuronal, and the later ones predominantly glial. How the restriction of cortical ventricular zone cells from multipotency to unipotency occurs (perhaps through oligopotent intermediates¹⁷) remains to be seen. Interestingly, we did not observe any neuron–oligodendrocyte stem cell clones, although small neuron–oligodendrocyte clones have been described in retroviral studies¹⁵, which perhaps represents a restriction in the development of stem cells.

Thus, as in neural crest^{6,18}, there may be a haemopoietic-like mechanism operating in the cerebral cortex. Is this model relevant to other regions of the CNS? Rare, large, neuron–glial clones have been described in cultures of septal region¹⁹ and striatum treated with epidermal growth factor²⁰, and the fact that similar cells have been extracted from adult striatum²¹ suggests self-renewal. In chick hindbrain, most clones are single-type, suggesting the presence of restricted progenitor cells²². Developing murine retina and chick spinal cord contain multipotential cells, but lack progenitors restricted to give one class of retinal or spinal cell^{23–26}. It appears then, that different regions of the CNS may use different strategies to generate cell diversity, although further studies may reveal common mechanisms. □

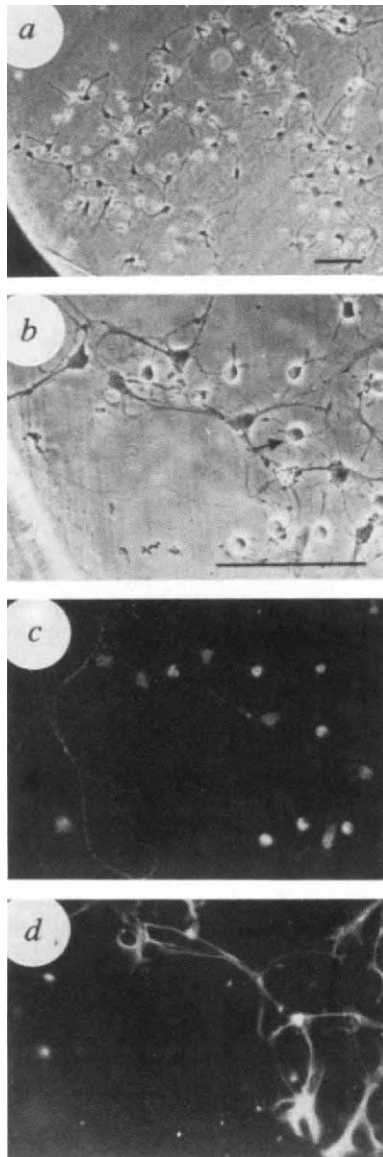


FIG. 3 The growth of one of the progeny of a stem cell after subcloning. *a*, Low-power phase micrograph of a portion of culture at 14 days to show that the subcloned cell produced numerous progeny. *b*, Phase micrograph showing the variety of morphologies present in this clone: oligodendroglial-like cells are clearly visible (single arrow). *c*, On staining with RT97, neurofilament-positive processes are seen in this region of the clone. *d*, GFAP-positive astrocytes are also present. A single cell from E14 ventricular zone was plated into a Terasaki well and grown under conditions described for Fig. 1. After 4 days the clone consisted of 26 cells and these were removed from the well by 0.5% trypsin plus 1 mM EDTA and replated as single cells in Terasaki wells under the same culture conditions as the initial single cell. Scale bar, 100 μ m.

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Neurotrophin-6 is a new member of the nerve growth factor family

Rudolf Götz^{*†}, Reinhard Köster^{*},
Christoph Winkler[‡], Friedrich Raulf^{‡§},
Friedrich Lottspeich^{||}, Manfred Scharlt[‡]
& Hans Thoenen^{*}

^{*} Max-Planck-Institute for Psychiatry, Department of Neurochemistry, Am Klopferspitz 18A, D-82152 Martinsried, Germany

[‡] Biozentrum der Universität Würzburg, Department of Physiologische Chemie I, Am Hubland, D-97074 Würzburg, Germany

^{||} Max-Planck-Institute for Biochemistry, Am Klopferspitz 18A, D-82152 Martinsried, Germany

DURING vertebrate development, many neurons depend for survival and differentiation on their target cells^{1–3}. The best documented mediator of such a retrograde trophic action is the neurotrophin nerve growth factor (NGF)⁴. NGF and the other known members of the neurotrophin family, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) are conserved as distinct genes over large evolutionary distances^{4–6}. Here we report the cloning of neurotrophin-6 (NT-6), a new member of this family from the teleost fish *Xiphophorus*. NT-6 distinguishes itself from the other known neurotrophins in that it is not found as a soluble protein in the medium of producing cells. The addition of heparin (but not chondroitin) effects the release of NT-6 from cell surface and extracellular matrix molecules. Recombinant purified NT-6 has a spectrum of actions similar to NGF on chick sympathetic and sensory neurons, albeit with a lower potency. NT-6 is expressed in the embryonic valvula cerebelli; expression persists in some adult tissues. The interaction of NT-6 with heparin-binding molecules may modulate its action in the nervous system.

Neurotrophin-6 was cloned from a genomic library of the platyfish *Xiphophorus maculatus* during our attempts to clone

TABLE 1 Amino-acid identity in per cent of fish NT-6 with other members of the nerve growth factor protein family

NGF, fish	61	NT-3, fish	52
NGF, human	56	NT-3, human	46
BDNF, fish	48	NT-4, frog	39
BDNF, human	48	NT-4/5, human	38

Note that for salmon NT-3 only 44 amino acids are known.

the fish NGF gene⁴. Overlapping genomic clones (Fig. 1a) were identified that hybridized to the mouse NGF probe under low-stringency hybridization conditions. DNA sequencing of the hybridizing region predicted a single long open reading frame of 858 base pairs encoding a polypeptide related to the known neurotrophins. Sequence alignments (Table 1) to the other known neurotrophin sequences in fish, including the complete NGF and BDNF sequences of *Xiphophorus*⁴, a partial NT-3 sequence of salmon⁶, and the sequences of neurotrophins from other vertebrate species suggested that it did not represent a homologous molecule in *Xiphophorus* of any known neurotrophin but rather a new member of the neurotrophin gene family. Accordingly, this factor was termed neurotrophin-6 (NT-6).

The deduced structure of the NT-6 precursor of 286 amino acids (predicted M_r of 31,424) is in accordance with the features of all known neurotrophins (Fig. 1b). (1) A hydrophobic domain at the N terminus with the characteristics of a signal peptide (amino acids 1–19). (2) A pro-region (amino acids 20–142) containing basic motifs (R-X-K/R-R at residues 63–66 and 139–142), necessary for proteolytic cleavage of precursor proteins into their mature, secreted forms⁷. (3) An amino-acid sequence at the carboxy half of the precursor starting at lysine 143 which encodes the mature NT-6 of 143 residues (M_r 15,968, pI 10.8; see also below the data on the recombinant protein). (4) Thirty-four residues, including the six cysteines conserved in all neurotrophins known so far, are also conserved in NT-6 suggesting that NT-6 shares a common tertiary structure with other neurotrophins; these residues are thought to be involved in the correct folding of the neurotrophins on the basis of the X-ray diffraction structure of NGF⁸. However, a distinguishing feature of NT-6 within the neurotrophin family is the presence of a 22 residue insert between the second and third conserved cysteine containing domain (Fig. 1b). This segment contains six basic and eight bend-inducing glycine⁹ residues.

Analysis of NT-6 gene expression during embryonic development by northern blotting revealed a transcript of 1.4 kilobases (kb) from organogenesis onwards, in 8-day-old fish and continuing expression of this transcript in adult brain (Fig. 2a). NT-6 was also expressed in adult gill, liver and eye with weak expression in skin, spleen, heart and skeletal muscle (Fig. 2a). *In situ* hybridization in consecutive serial sections of embryos revealed expression in the valvula cerebelli, a rostral protrusion of the teleostean cerebellum under the midbrain tectum¹⁰ (Fig. 2b–d).

Recombinant NT-6 was obtained from a rabbit kidney cell line (RK13) infected with a vaccinia virus expression vector that contained the fish NT-6 gene inserted in its genome (Fig. 3a), and from insect cells infected with a baculovirus expression vector containing the NT-6 gene, respectively. Purified NT-6 from both preparations showed survival activity on embryonic chick neurons prepared from different ganglia. NT-6 promotes the survival of sympathetic and sensory dorsal root ganglion (DRG) neurons to the same extent as NGF, albeit with a much lower specific activity (Fig. 3b). It had no survival effect on ciliary and nodose neurons (Fig. 3b), which also do not respond to NGF. Thus, the spectrum of responsive neurons is similar to that of NGF. A monoclonal antibody that inhibited the activity of NGF showed no inhibition of the survival activity of NT-6 (Fig. 3c). The high concentrations of NT-6 needed (50 ng ml⁻¹ for half-maximal survival) to obtain neuronal survival with chick

[†] To whom correspondence should be addressed at present address: Neurologische Klinik, Universität Würzburg, Josef-Schneider-Straße 11, D-97080 Würzburg, Germany.

[§] Present address: Sandoz Pharma AG, Präklinische Forschung 386/607, Postfach, CH-4002 Basel, Switzerland.

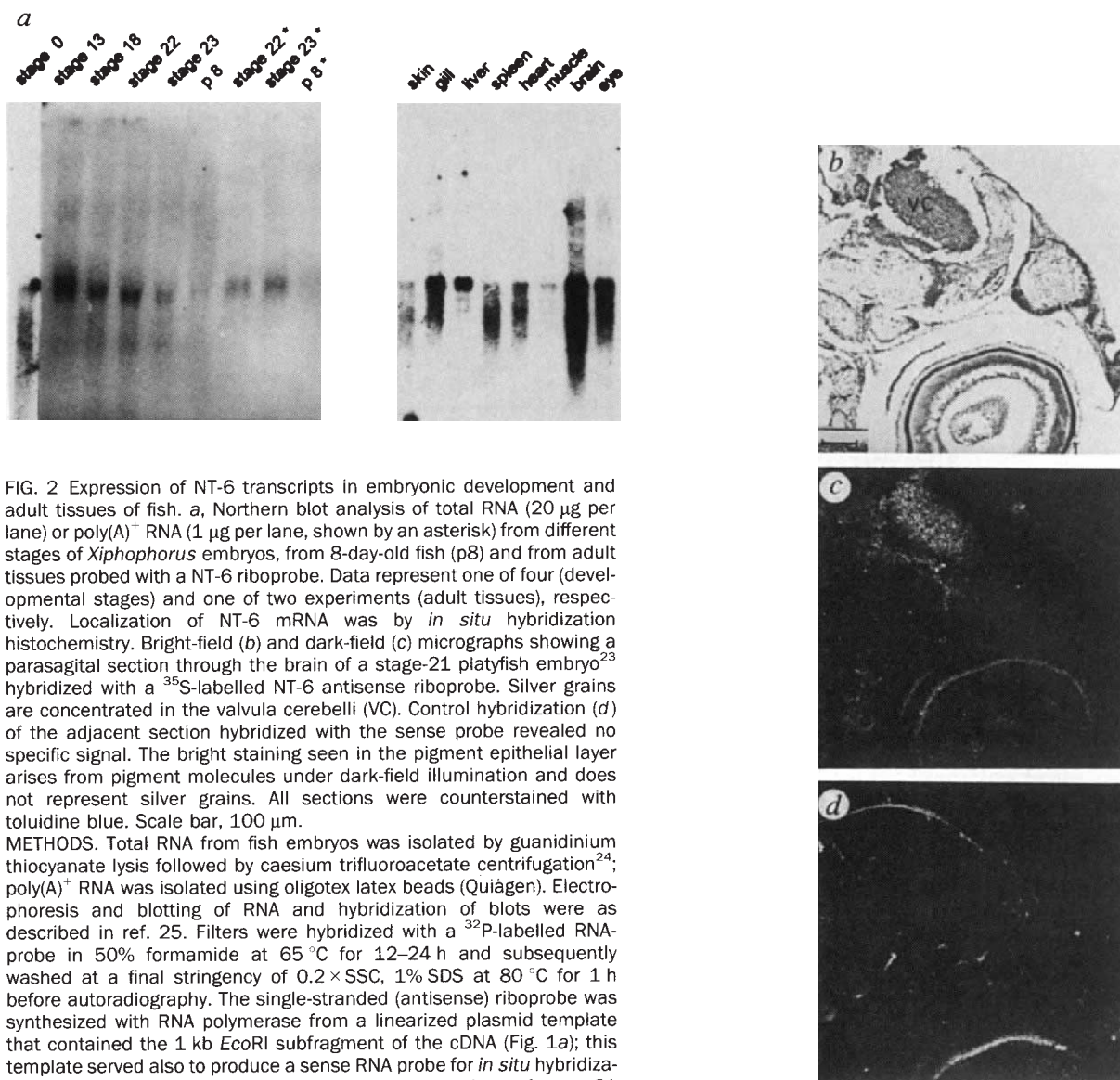


FIG. 2 Expression of NT-6 transcripts in embryonic development and adult tissues of fish. *a*, Northern blot analysis of total RNA (20 μ g per lane) or poly(A)⁺ RNA (1 μ g per lane, shown by an asterisk) from different stages of *Xiphophorus* embryos, from 8-day-old fish (p8) and from adult tissues probed with a NT-6 riboprobe. Data represent one of four (developmental stages) and one of two experiments (adult tissues), respectively. Localization of NT-6 mRNA was by *in situ* hybridization histochemistry. Bright-field (*b*) and dark-field (*c*) micrographs showing a parasagittal section through the brain of a stage-21 platyfish embryo²³ hybridized with a ³⁵S-labelled NT-6 antisense riboprobe. Silver grains are concentrated in the valvula cerebelli (VC). Control hybridization (*d*) of the adjacent section hybridized with the sense probe revealed no specific signal. The bright staining seen in the pigment epithelial layer arises from pigment molecules under dark-field illumination and does not represent silver grains. All sections were counterstained with toluidine blue. Scale bar, 100 μ m.

METHODS. Total RNA from fish embryos was isolated by guanidinium thiocyanate lysis followed by caesium trifluoroacetate centrifugation²⁴; poly(A)⁺ RNA was isolated using oligotex latex beads (Quiagen). Electrophoresis and blotting of RNA and hybridization of blots were as described in ref. 25. Filters were hybridized with a ³²P-labelled RNA probe in 50% formamide at 65 °C for 12–24 h and subsequently washed at a final stringency of 0.2 $\times$ SSC, 1% SDS at 80 °C for 1 h before autoradiography. The single-stranded (antisense) riboprobe was synthesized with RNA polymerase from a linearized plasmid template that contained the 1 kb *Eco*RI subfragment of the cDNA (Fig. 1a); this template served also to produce a sense RNA probe for *in situ* hybridization. *In situ* hybridizations were done on 7- μ m sections of stage-21 *Xiphophorus* embryos that had been cut from paraffin-embedded specimens. Glass slides containing adjacent serial sections were hybridized with either sense or antisense NT-6 riboprobes using the *in situ* hybridization protocol described in ref. 26. Riboprobes were labelled with ³⁵S-UTP and used without alkaline hydrolysis.

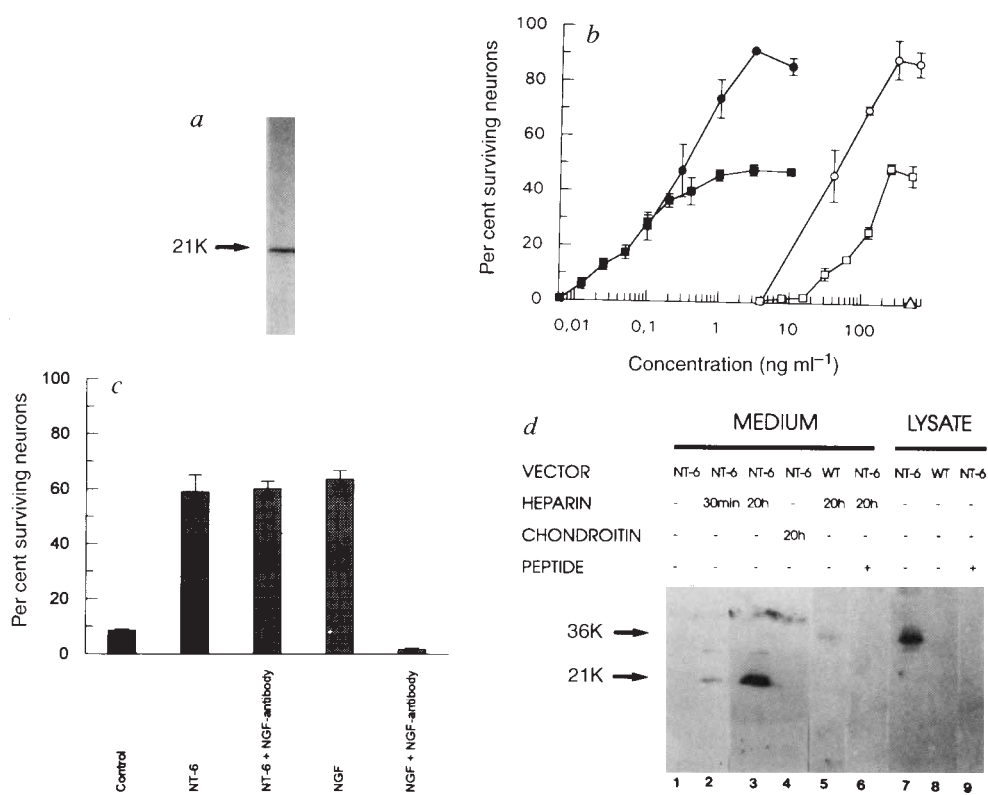
The cell lysate, however, contained a 36K antigen (Fig. 3d, lane 7) that was not present in cells infected with wild-type virus (lane 8) and that could be eliminated by preincubation of the antiserum used to detect the protein on western blots with the corresponding peptide antigen (lane 9). Thus, this protein is probably the NT-6 precursor.

The absence of NT-6 in the conditioned medium might be due to its sequestration at the cell surface and/or matrix. Proteoglycans have been found to be the binding sites of many growth factors with different heparin binding domains¹¹. The sequence of NT-6 contains a motif 'inserted' between the second and third cysteine residues with the characteristics of a heparin-binding domain formed by the positively charged amino acids (Fig. 1b). Interestingly, this basic domain of NT-6 would be expected to be on the protein surface on the basis of the X-ray structure of NGF⁸ and therefore would probably be available for interaction with anionic charges of heparin. This hypothesis was supported by the fact that the addition of heparin to the expressing cells

resulted in the appearance of a band of 21K in the medium (Fig. 3d, lanes 2 and 3). This form of NT-6 represents the mature molecule (Fig. 3a) which we propose is bound to the cell surface and/or extracellular matrix. Addition of chondroitin sulphate, another glycosaminoglycan, did not release NT-6, showing the specificity of heparin (Fig. 3d, lane 4). Further support of the heparin-binding capacity of NT-6 was obtained by heparin agarose chromatography. NT-6 eluted at 700 mM sodium chloride (data not shown); similar salt concentrations (600–1,100 mM) are required for the elution from heparin of some other heparin-binding molecules such as heparin-binding epidermal growth factor and the membrane-associated forms of platelet-derived growth factor^{12–15}.

The most likely explanation for these observations is the binding of NT-6 to proteoglycans of the cell surface and/or matrix. Whether heparin protects secreted soluble NT-6 from proteolytic degradation must be investigated. It also remains to be established whether NT-6 requires the presence of heparin or

FIG. 3 Expression and biological activity of NT-6. **a**, SDS-PAGE analysis of purified NT-6 (2 µg of protein loaded) stained with Coomassie brilliant blue. N-terminal sequencing of the NT-6 obtained after the final reversed phase HPLC chromatography yielded the sequence KAVSHTM?R (single-letter amino-acid code) confirming the proteolytic cleavage on the carboxyl side of the subtilisin-like serine protease PACE/furin cleavage motif⁷ (Fig. 1b). **b**, Effect of NT-6 on the survival of sensory neurons prepared from the DRG (white squares) or nodose ganglia (white triangle) of 8-day-old chick embryos (E8) and sympathetic E10 neurons (white circles). Dose-response curves of the survival effect of NT-6 in comparison to that of mouse NGF on sympathetic (black circles) and sensory (black squares) neurons are shown. **c**, Bar graph showing the effects on the survival of sensory neurons in the presence of NT-6 (500 ng ml⁻¹) or NGF (10 ng ml⁻¹) with or without the anti-NGF antibody (clone 27-21; Boehringer-Mannheim). **d**, Heparin requirement to release NT-6 from producing cells into the medium. Western blot analysis of medium and cell lysates from cells infected with a NT-6 recombinant vaccinia virus or a wild-type virus (WT) vectors. Heparin or chondroitin (100 µg ml⁻¹) were added as indicated for a period of 20 h or for 30 min at the end of the 20 h incubation period. Medium from 2 × 10⁵ cells and the lysate from 4 × 10³ cells were loaded. The amount of precursor per cell is about 50-fold greater than the 21K mature protein and therefore the latter is not detectable on the blot. A control blot was developed (lanes 6 and 9) where the anti-serum was preincubated with NT-6 peptide (1 µg ml⁻¹).



vector and the purification are to be published in detail elsewhere. N-terminal sequencing of NT-6 was done on a gas/liquid-phase sequencer 477A from Applied Biosystems equipped with an on-line 120A phenylthiohydantoin analyser using the conditions given by the manufacturer. Proteins were separated by 0.1% SDS, 15% polyacrylamide electrophoresis²⁸, transferred to nitrocellulose filter membranes and the immune complexes were detected with chemiluminescence. For the detection of NT-6 protein we raised polyclonal antibodies against a peptide corresponding to the N terminus of the mature NT-6 sequence (KAVSHTMHRGEYSVC, see Fig. 1b). Neuronal survival assays were as described previously^{29,30}. Results are the mean ± s.d. of the per cent survival for 6 wells. All experiments were done at least twice.

a specific heparan sulphate proteoglycan for optimal biological activity in addition to a signal-transducing receptor, as is the case with the fibroblast growth factors^{16,17}. The binding of NT-6 to the cell surface and extracellular matrix might spatially restrict the action of this molecule and might also be the basis for an extracellular storage form as has been shown for

fibroblast growth factor bound to heparan sulphate proteoglycans^{18,19}. Small aquarium fish can be used as model systems combining the power of genetic approaches with experimental embryology²⁰⁻²² to study the physiological role of this new property of NT-6 in the development and function of the nervous system. □

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Operons as a common form of chromosomal organization in *C. elegans*

Diego A. R. Zorio, Niansheng Nick Cheng,
Thomas Blumenthal & John Spieth

Department of Biology, Indiana University, Bloomington,
Indiana 47405, USA

ALTHOUGH eukaryotic genes are usually transcribed individually, at least a few *Caenorhabditis elegans* genes appear to be transcribed polycistronically in clusters resembling bacterial operons¹. The spliced leader SL2 (ref. 2) is specific for *trans*-splicing to downstream genes in these operons¹. In addition, many *C. elegans* pre-mRNAs are *trans*-spliced to SL1 (ref. 3) near the 5' ends of pre-mRNAs^{4,5}. Because operons have not previously been found in higher eukaryotes, we have investigated how widespread they are in the *C. elegans* genome. We identified gene

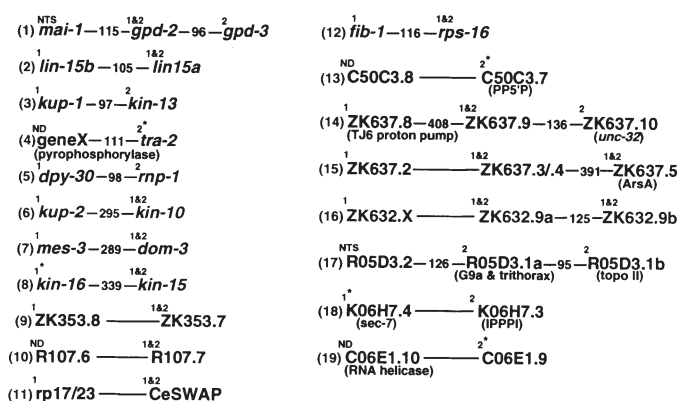


FIG. 1 *C. elegans* operons. Genes in closely spaced clusters in which downstream gene products receive SL2 are included. Distances between genes are in bp. *Trans*-splicing to SL1, SL2, or both, is indicated by a superscript 1, 2 or 1 & 2. NTS indicates not *trans*-spliced and ND indicates not determined. An asterisk indicates that this gene was not tested for both spliced leaders. Operon (1) *mai-1* is homologous to vertebrate mitochondrial ATPase inhibitor¹; *gpd-2* and *gpd-3* are nearly identical and encode glyceraldehyde phosphate dehydrogenase¹²; (2) *lin-15a* and *lin-15b* function in cell-cell signalling events in development of the vulva^{8,9}; (3) *kin-13* encodes protein kinase C1 (ref. 13); (4) *tra-2* is in the sex-determination pathway¹⁴; *geneX* is similar to a family of pyrophosphorylases (P. Kuwabara, personal communication); (5) *dpy-30* is required for dosage compensation; *rnp-1* contains an RRM motif found in RNA-binding proteins and a zinc-finger of the retroviral RNA packaging class (D. Hsu and B. Meyer, personal communication); (6) *kin-10* encodes the β -subunit of casein kinase II (ref. 15); (7) *mes-3* is a maternal effect sterile gene (ref. 16, and J. Paulsen and S. Strome, personal communication); (8) *kin-15* and *kin-16* encode two similar transmembrane protein tyrosine kinases¹⁷; (11) CeSWAP encodes a homologue to the *Drosophila* suppressor of white apricot gene; *rp17/23* encodes a ribosomal protein (K. Van Doren, personal communication); (12) *fib-1* and *rps-16* are *C. elegans* homologues of the fibrillar and ribosomal protein S16 genes; (13) C50C3.7 encodes an inositolpolyphosphate-5'-phosphatase (M. Perry, personal communication); (14) ZK637.8 is a TJ6/proton pump homologue; ZK637.10 is similar to glutathione reductase and may be the *unc-32* gene (N. Pujol and D. Thierry-Mieg, personal communication); (15) ZK637.5 is an arsenic pump ATPase homologue; (17) R05D3.1a is related to the vertebrate G9a and *Drosophila trithorax* genes; R05D3.1b is DNA topoisomerase II (M. Perry, personal communication); (18) K06H7.4 has homology to the yeast *sec7* gene; K06H7.3 has homology with isopentenyl diphosphate δ -isomerase; (19) C06E1.10 has homology with ATP-dependent RNA helicase. None of the others show homology to any known genes.

clusters using the extensive data generated by the genome project^{6,7} and tested seven for *trans*-splicing specificity. All were found to fit expectations for polycistronic transcription. In addition, we surveyed reported *C. elegans* genes for *trans*-splicing specificity. Both methods indicate that the pre-mRNAs of about 70% of *C. elegans* genes are *trans*-spliced and as many as a quarter are transcribed in operons.

Table 1 shows the sequenced cosmids^{6,7} used to estimate the frequency of operons. We tested seven clusters (Fig. 1: operons 9, 10, 14, 15, 16, 17 and 18) by reverse transcription combined with polymerase chain reaction (RT-PCR) to determine whether the predicted genes were transcribed and the mRNAs *trans*-spliced. The data for four of the clusters are shown in Fig. 2. In none of the clusters tested was the messenger RNA of the first gene *trans*-spliced to SL2. In some clusters it was *trans*-spliced to SL1 but in others it was not *trans*-spliced. In contrast, the mRNAs from all downstream genes were *trans*-spliced exclusively to SL2 or to a mixture of SL2 and SL1. Our earlier work on *C. elegans* operons showed that the distances between five of the six gene pairs were very close to 100 base pairs (bp), with

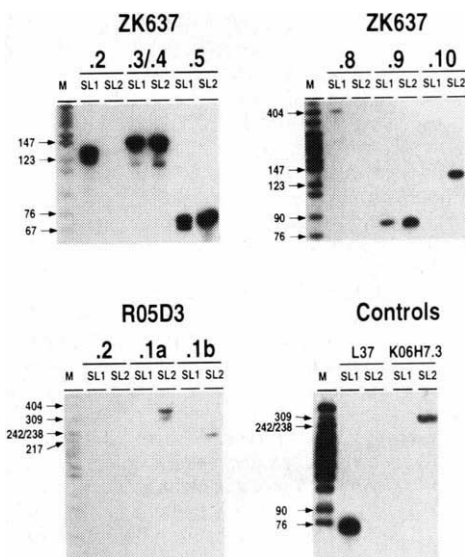


FIG. 2 *Trans*-splicing to transcripts of four operons. The sequences of cosmids from the *C. elegans* genome project were searched for potential operons (Table 1 legend) and tested for *trans*-splicing by RT-PCR. Predicted genes are designated by the cosmid name and a number. (ZK632.X was not predicted by GENEFINDER to be a gene; R05D3.1a and R05D3.1b, as well as ZK632.9a and ZK632.9b, were each predicted to be one gene, and ZK637.3/4 was predicted to be two). The 5'-most gene in an operon is on the left in each panel, followed on the right by the central gene and then the 3'-most gene. The 5'-most gene in the K05H7 operon was not tested. The *C. elegans* homologue of the ribosomal-protein L37 was used as an SL1-specific control and K06H7.3 was used as an SL2-specific control. Lanes M, size markers in base pairs. Total RNA was prepared and reverse transcription, PCR (25 cycles) polyacrylamide gel electrophoresis, electroblotting and hybridization were all done as described^{1,4}. Upstream PCR primers were either SL1-20 or SL2-20 (ref. 1). Downstream primers, and their positions in the GenBank sequences, used in reverse transcription and PCR are complementary to the nucleotide positions given in parentheses: ZK637.2 (3,898 to 3,882); ZK637.3/4 (6,392 to 6,372); ZK637.5 (11,169 to 11,150); ZK637.8 (22,244 to 22,226); ZK637.9 (28,240 to 28,222); ZK637.10 (29,910 to 29,895); R05D3.2 (22,788 to 22,771); R05D3.1a (26,131 to 26,116); R05D3.1b (31,630 to 31,613); L37 (42 to 34; GenBank accession number M79655); K06H7 (8,565 to 8,546). The probe oligonucleotides for each gene are: ZK637.2 (3,807 to 3,826); ZK637.3/4 (6,296 to 6,318); ZK637.5 (11,144 to 11,128); ZK637.8 (22,199 to 22,218); ZK637.9 (28,200 to 28,219); ZK637.10 (29,838 to 29,858); R05D3.2 (22,748 to 22,767); R05D3.1a (26,040 to 26,059); R05D3.1b (31,654 to 31,580); L37 (33 to 3); K06H7.3 (8,204 to 8,222).

TABLE 1 Scanned cosmids

Cosmid	Base pairs	Accession number	SL1 <i>trans</i> -spliced	SL2 <i>trans</i> -spliced	Non- <i>trans</i> -spliced	Number of operons	Genes per operon
B0303	41,071	M77697	5	0	8	0	—
B0464	10,909	Z19152	7	0	0	0	—
B0523	14,334	L07143	2	0	3	0	—
C06E1	40,216	L16559	4	2	4	2†	2, 2
C14B9	43,492	L15188	4	1	0	1	2
C29E4	40,050	L23651	5	1	3	1	2
C30A5	27,744	L10990	5	1	3	1	2
C38C10	34,193	Z19153	3	0	2	0	—
C40H1	27,271	Z19154	4	0	2	0	—
C50C3	44,733	L14433	4	7	0	3†,‡	2, 3, 5
F02A9	26,242	Z19555	4	1	0	1	2
F09G8	41,645	L11247	3	0	5	0	—
F22B7	40,222	L12018	7	0	3	0	—
F54F2	39,573	L23645	6	1	1	1	2
F54G8	31,613	Z19155	2	0	2	0	—
F58A4	38,000	Z22179	5	2	3	1	3
F59B2	43,782	Z11505	6	1	4	1	2
K06H7*	22,273	L15314	4	2	2	2†	2
K07D8	3,607	L16679	1	0	0	0	—
K11H3	33,000	Z22180	3	0	2	0	—
K12H4	38,582	L14331	5	3	0	1	4
R05D3*	38,810	L07144	5	2	3	1	3
R08D7	27,368	Z12017	5	1	1	1	2
R107*	40,870	Z14092	5	2	0	2†	2, 2
T02C1	10,308	Z19156	0	0	1	0	—
T23G5	26,926	Z19158	5	1	0	1	2
ZC84	38,955	Z19157	1	0	3	0	—
ZC97	4,166	L14714	1	1	0	1	2
ZK353*	24,916	L15313	4	1	3	1†	2
ZK370	37,675	M98552	8	0	0	0	—
ZK632*	36,000	Z22181	8	2	2	1	3
ZK637*	40,699	Z11115	2	4	1	2	3, 3
ZK643	39,534	Z11126	4	0	4	0	—
ZK652	36,052	L14429	5	1	2	1	2
ZK686	11,435	L17337	2	0	1	0	—
ZK688	36,977	L16621	4	2	2	2	2
ZK1098	37,310	Z22176	3	5	1	3	2, 2, 4

The GENEFINDER analysis of 30 randomly chosen cosmids was examined for clusters of closely spaced genes in the same orientation. GENEFINDER predicts genes beginning with the initiator methionine codon and ending with a translation termination codon, as well as introns within genes. Sequences upstream of initiator methionines were examined for *trans*-splice sites and sequences downstream of termination codons were searched for potential polyadenylation signals. Clusters oriented in the same direction in which downstream genes had potential *trans*-splice sites, upstream genes contained potential polyadenylation signals, and the distances between genes were small, were chosen for further investigation.

* Cosmids with clusters that have been tested for *trans*-splicing.

† cDNA clones have been isolated containing on their 5' ends either part of the SL2 leader sequence in the case of the downstream genes C06E1.9 (GenBank accession number Z14710), ZK353.7 (accession number Z14728)¹¹ and C50C3.7 (M. Perry, personal communication), or part of the SL1 leader sequence in the case of the upstream gene K06H7.4 (accession number M89330).

‡ Only one of the clusters (C50C3.8/.7, K06H7.4/.3 and R107.6/.7) on these cosmids was analysed.

the other being about 300 bp¹. We determined the spacing between genes in some of the newly identified clusters by identifying the 3' ends of upstream genes and the *trans*-splice sites of the downstream genes, and a few others have been determined independently. Eleven of the gene pairs are ~100 bp apart; five others are ~3–400 bp apart. The significance of this asymmetric distribution of gene spacings is unknown.

Figure 1 shows all genes for which there are data indicating that they are part of an operon. Also shown are the *trans*-splicing specificities, distances between genes, and any known similarities to other genes. The mRNAs from 16 downstream genes (13 from cosmids analysed here) that are in closely spaced clusters with the same orientation were tested for *trans*-splicing; all 16 were found to be *trans*-spliced either to SL2 or to a mixture of the two SLs. In contrast, all genes that were not downstream genes in clusters were *trans*-spliced exclusively to SL1 or not *trans*-spliced at all. It seems fair to conclude that the presence of a cluster of closely spaced genes in the same orientation is indicative of a polycistronic transcription unit.

We also identified two new SL2-containing mRNAs from a cDNA library specific for SL2 *trans*-spliced mRNAs (N.N.C.

and T.B., unpublished results). The gene for one is the last gene in a three-gene cluster within the sequenced cosmid ZK632 (Fig. 1, operon 16). The mRNAs of both downstream genes are *trans*-spliced to SL2. The other clone encodes the *C. elegans* homologue for the ribosomal protein S16 (Fig. 1, operon 12). The sequence of a genomic clone selected with this cDNA shows that just 116 bp upstream of the *rps-16 trans*-splice site is the *C. elegans* homologue of the fibrillarlin gene (N.N.C. and T.B., unpublished results). A closely spaced upstream gene has now been found in eight cases where a gene's mRNA was originally observed to be *trans*-spliced to SL2. Thus it appears that *trans*-splicing of SL2 to an mRNA indicates that it is from a downstream gene in an operon.

Table 2 shows the percentage of genes whose mRNAs are predicted to be *trans*-spliced to SL1 and SL2 based on two independent methods. First, we analysed 37 cosmids containing ~2 megabases of sequenced DNA, or about 2% of the *C. elegans* genome, for the presence of *trans*-splice sites and for gene clusters. Of the 266 genes examined, 73% were predicted to be *trans*-spliced and 26% in operons. This includes the 5'-most genes in clusters, some of which are predicted to be SL1 *trans*-spliced

TABLE 2 Surveys of *trans*-splicing to *C. elegans* gene products

	Number of genes	Not <i>trans</i> -spliced (%)	<i>Trans</i> -spliced to SL1 (%)	SL2 (%)
Cosmid genes	266	27	57	16
Reported genes	115	30	57	13

and some not *trans*-spliced, and downstream genes, which are predicted to be SL2 *trans*-spliced. Second, we surveyed reported *C. elegans* genes for *trans*-splicing to SL1 exclusively, or to SL2 (either alone or to a mixture of the two SLs). Although each method has weaknesses, the results of the two methods are surprisingly similar, indicating that these independent estimates are probably an accurate reflection of the percentage of mRNAs not *trans*-spliced and *trans*-spliced to the two spliced leaders. Hence, we believe that 70% is a reasonable estimate of the fraction of genes whose products are *trans*-spliced, and that 26% is a reasonable estimate of the percentage found in operons.

Are operons in *C. elegans* a way of coordinating expression of genes with related functions, as they are in bacteria? Clearly the *lin-15A* and *lin-15B* proteins collaborate in a single process^{8,9}. In addition, arguments can be made that it makes sense to co-regulate the products of the *mai-1* operon^{1,10}. Furthermore, the *fib-1/rps-16* operon may help coordinate ribosomal RNA and ribosomal protein biogenesis. But in most cases it is not clear

that genes in the same operon are functionally related, perhaps because we do not know all the functions of particular genes. On the other hand, it may be that operons are merely a consequence of strong pressure for a small genome. If so, then genes may be in the same operon simply because the cost of inappropriate expression is lower than the benefit of a reduced genome size. □

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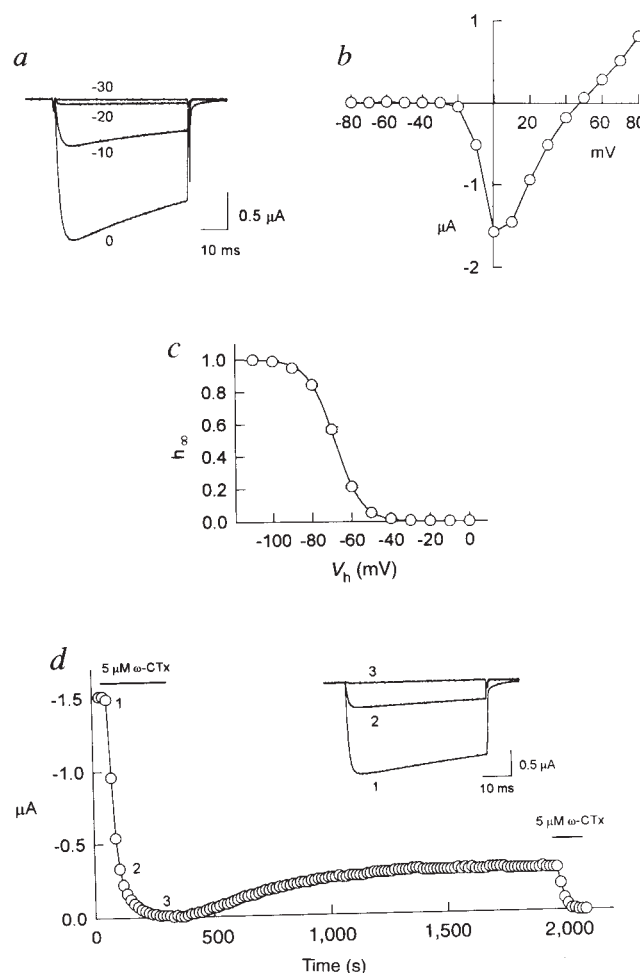
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Structural determinants of the blockade of N-type calcium channels by a peptide neurotoxin

Patrick T. Ellinor, Ji-Fang Zhang, William A. Horne* & Richard W. Tsien†

Department of Molecular and Cellular Physiology, Stanford University Medical Center, Stanford, California 94305, USA
* Neurex Corporation, Menlo Park, California 94025, USA

NEUROTOXINS that selectively block Na^+ , K^+ or Ca^{2+} channels have provided valuable information about the functional diversity of the voltage-gated channel superfamily¹. For Ca^{2+} channels, a variety of toxins have been found to block individual channel types^{2,3}. The best-known example is ω -conotoxin-GVIA, a member of a large family of peptide toxins derived from venomous cone snails^{2,3}, which potently and selectively blocks N-type Ca^{2+} channels^{4–9}, allowing their purification^{10,11}, cellular localization^{12,13}, and the elucidation of their roles in Ca^{2+} entry¹⁴, neurotransmitter release^{15,16} and neuronal migration¹⁷. In contrast to Na^+ and K^+ channels, little is known about the molecular features that underlie Ca^{2+} -channel susceptibility to toxin block; it is also unknown whether block occurs by direct physical occlusion³ or an action on channel gating¹⁸. Here we describe structural determinants of the N-type Ca^{2+} channel's interaction with ω -conotoxin-GVIA. When chimaeras combining individual motifs from the N-type channel and from a channel insensitive to ω -conotoxin-GVIA were expressed in *Xenopus* oocytes, each of the four motifs appeared to contribute to interaction with the toxin. The most dramatic effects on toxin interactions were seen at a single cluster of residues in the large putative extracellular loop



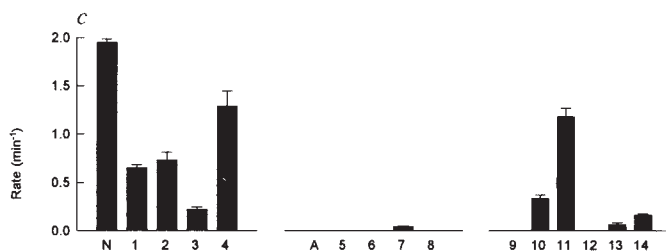
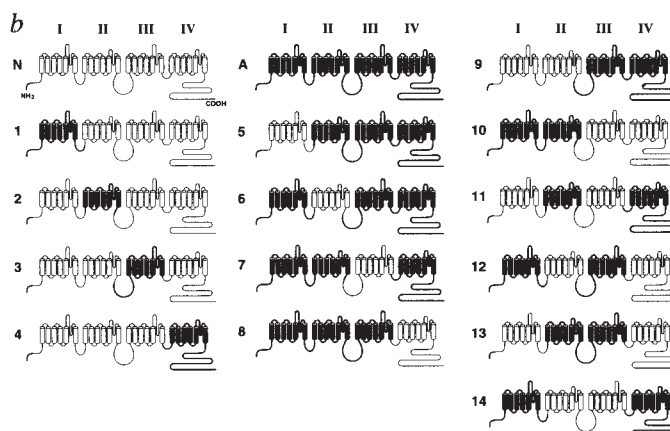
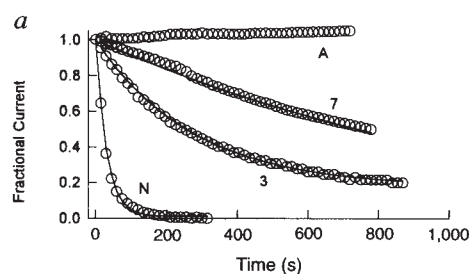
† To whom correspondence should be addressed.

FIG. 2 The contribution of individual motifs to the ability of Ca^{2+} channels to interact with a conotoxin. *a*, Time course of blockade of wild-type channels (N-type and class A) and chimaeras NA3 and NA7 by exposure to 5 μM ω -CTx-GVIA. The chimaeras illustrate the importance of motif III. The current amplitudes have been normalized to that at the start of the toxin application. Smooth curves are single exponential fits (N-type, $\tau = 29.5$ s; NA3, $\tau = 272.8$ s; NA7, $\tau = 1,178$ s). *b*, Scheme of chimaeric channels NA1 to NA14, as derived from α_{1B} subunits encoding the ω -CTx-GVIA-sensitive N-type channel (N) and α_{1A} subunits encoding toxin-insensitive channels (A). *c*, Pooled data showing the average rate of onset of blockade following exposure to 5 μM ω -CTx-GVIA for each of the mutants in *b*.

METHODS. The chimaeras between the N-type channel (N) and the class A channel (A) were designated NA1–14 and were constructed as follows: NA1: (1) chimaeric PCR primer starting at an *AgeI* restriction enzyme digestion site of N(1,452N) was used to amplify a fragment of BI-5 (see below) from 1,232–1,603; (2) the product of (1) was ligated into N at *EcoRV* (polylinker) and *AgeI* (1,452N) sites; (3) the *EcoRV*–*NsiI* fragment of BI-5 (polylinker–1,232) was ligated into (2). NA2: (1) *HindIII* fragment of BI-5 (5' end–2,502A) was subcloned into pBluescript SKII⁺; (2) chimaeric PCR primers from *XhoI* (1,688) and *NsiI* (1,232) of BI-5 were used to amplify a region of N from 1,082–1,522 and ligated into (1); (3) the *Bsp106I* (polylinker)–*BglII* (1,462N) fragment of N was ligated into (2); (4) *HindIII* fragment of (3) (5' end–2,502B) into *HindIII* of N (5' end–2,339N). NA3: *HindIII* fragment of N (5' end–2,339N) into *HindIII* of NA14 (5' end–2,502A). NA4: (1) *AccI*–*XbaI* fragment of BI-2 (4,797–3' end) into *AccI*–*XbaI* of N (4,337–7,321); (2) *AccI* fragment of N (4,007–4,337) into (1). NA5: *HindIII* fragment of N (5' end–2,339) into *HindIII* of BI-5 (5' end–2,502). NA6: *HindIII* fragment of BI-2 (5' end–2,502) into *HindIII* of N (5' end–2,339). NA7: *Sall* fragment of NA2 (5' end–2,678N) into *Sall* of NA4 (5' end–2,678N). NA8: *HindIII* of NA1 (5' end–2,339N) into *HindIII* of NA14 (5' end–2,502A). NA9: *HindIII* fragment of NA2c (5' end–2,502B) into *HindIII* of NA14 (5' end–2,502A). NA10: *Sall* fragment of NA1 (5' end–2,678N) into *Sall* of NA-4 (5' end–2,678N). NA11: *HindIII* fragment of NA2c (5' end–2,502A) into *HindIII* of BI-5 (5' end–2,502). NA12: *HindIII* fragment of NA1 (5' end–2,339N) into *HindIII* of BI-5 (5' end–2,502). NA13: *Sall* fragment of NA6 (5' end–2,678N) into *Sall* of NA4 (5' end–2,678N). NA14: *AccI* fragment of BI-2 (5' end–4,797) into N (5' end–4,337). BI-5 is a modified version of the class A channel (BI-2) which contains a deletion of the *BamHI* fragment (7,612–3' end) and a *NsiI* site at 1,232.

FIG. 1 Functional properties of the N-type calcium channel activity expressed in *Xenopus* oocytes. Complementary RNA encoding the α_1 subunit was co-expressed with α_2 and β_1 subunits in *Xenopus* oocytes, and two-microelectrode voltage-clamp recordings were carried out 2–3 days after injection. *a*, Representative traces of Ba^{2+} currents carried by N-type channels. Holding potential, -80 mV; test potentials as indicated. *b*, Peak current–voltage relationship for the same experiment as in *a*. *c*, Steady-state inactivation curve. Smooth curve is a Boltzmann function, $h_\infty = [1 + \exp \{(V - V_{1/2})/k\}]^{-1}$, with $V_{1/2} = -68.4 \pm 0.3$ mV, $k = -6.68 \pm 0.11$ mV ($n = 5$). *d*, Effect of 5 μM ω -CTx-GVIA on the amplitude of the N-type channel current. Numbered points correspond to sweeps in the inset. The reversibility of toxin block was not always seen, possibly due to channel run-down, and was not analysed in detail.

METHODS. To obtain a cDNA for a human α_{1B} subunit, one million recombinants of a λ ZAP II human hippocampal cDNA library (Stratagene) were screened using a *HindIII* (712–2,288) and a *XhoI* (3,793–5,394) fragment of the rat α_{1B} subunit. Clone H20 (1,222–3' end) was obtained using standard procedures. Polymerase chain reaction (PCR) was used to amplify the remaining region (5' end–1,328) from mRNA prepared from IMR-32 cells. A fragment (5' end–513) of the class A channel was ligated to the 5' end of the N-type channel at a conserved *HincII* site (211 of N). cRNA was synthesized *in vitro* using T7 or SP6 polymerase. cRNA for each subunit was injected in approximately equimolar ratios (0.1 $\mu\text{g ml}^{-1}$ α_1 , 0.1 $\mu\text{g ml}^{-1}$ α_2 and 0.03 $\mu\text{g ml}^{-1}$ β_1); about 50 nl was injected per cell. Channel currents were recorded by a standard two-microelectrode voltage clamp using a Warner amplifier (OC-725 A). The bath solution was chloride-free, containing (in mM): $\text{Ba}(\text{OH})_2$, 5; tetraethylammonium-OH, 85; KOH, 2; HEPES, 5; pH 7.4 adjusted with methanesulphonic acid. Data were sampled at 10 kHz and filtered at 2 kHz. Leak and capacitance currents were subtracted off-line by a P/4 protocol. Voltage pulses were delivered every 15 s. The magnitude of the current was somewhat variable, but currents were usually >2 μA in size with 5 mM Ba^{2+} as the charge carrier, far larger than endogenous currents. The currents analysed here were always >0.5 μA .



between IIIS5 and IIHS, consistent with a direct pore-blocking mechanism. These results provide a starting point for delineating the architecture of the outer vestibule of the Ca^{2+} channel.

Mutational studies of the N-type channel have been hampered by the difficulty of expressing its α_1 subunit^{19,21} (α_{1B}) in *Xenopus* oocytes. To circumvent this problem, we made a complementary DNA construct encoding a channel with all the key features of N-type channels, but with the strong expression of the class A subunit (α_{1A})^{22,23}. Complementary RNA encoding the α_1 subunit was coexpressed with that for α_2 and β_1 subunits in *Xenopus* oocytes (Fig. 1 legend). Two-microelectrode voltage-clamp recordings carried out 2–3 days after injection revealed inward currents that were activated by high voltage and typically ≥ 2 μA with 5 mM external Ba^{2+} (Fig. 1a, b). The peak current rose most steeply with depolarization near -20 mV, reached maximal amplitude between 0 and $+10$ mV, and reversed at $+49.2 \pm 0.31$ mV (mean $\pm$ s.e.m., $n = 7$). As expected for N-type channels, the currents were susceptible to inactivation with steady depolarizations, with 50% inactivation at a holding potential, V_h , of -68.4 ± 0.3 mV ($n = 5$) (Fig. 1c). The Ca^{2+} channel current was rapidly blocked by the conotoxin ω -CTx-GVIA (5 μM , $\tau = 30.8 \pm 0.6$ s, $n = 4$; Fig. 1d). Currents in the absence of the toxin and during the onset of blockade were similar in waveform (Fig. 1d, inset). On the other hand, the current was unresponsive to 1 μM FPL64176 ($n = 3$), a potent agonist of L-type channels, or 100 nM ω -Aga-IVA ($n = 3$), a blocker of P- and Q-type channels. In each respect, the expressed current behaves as expected for an N-type Ca^{2+} channel. A somewhat

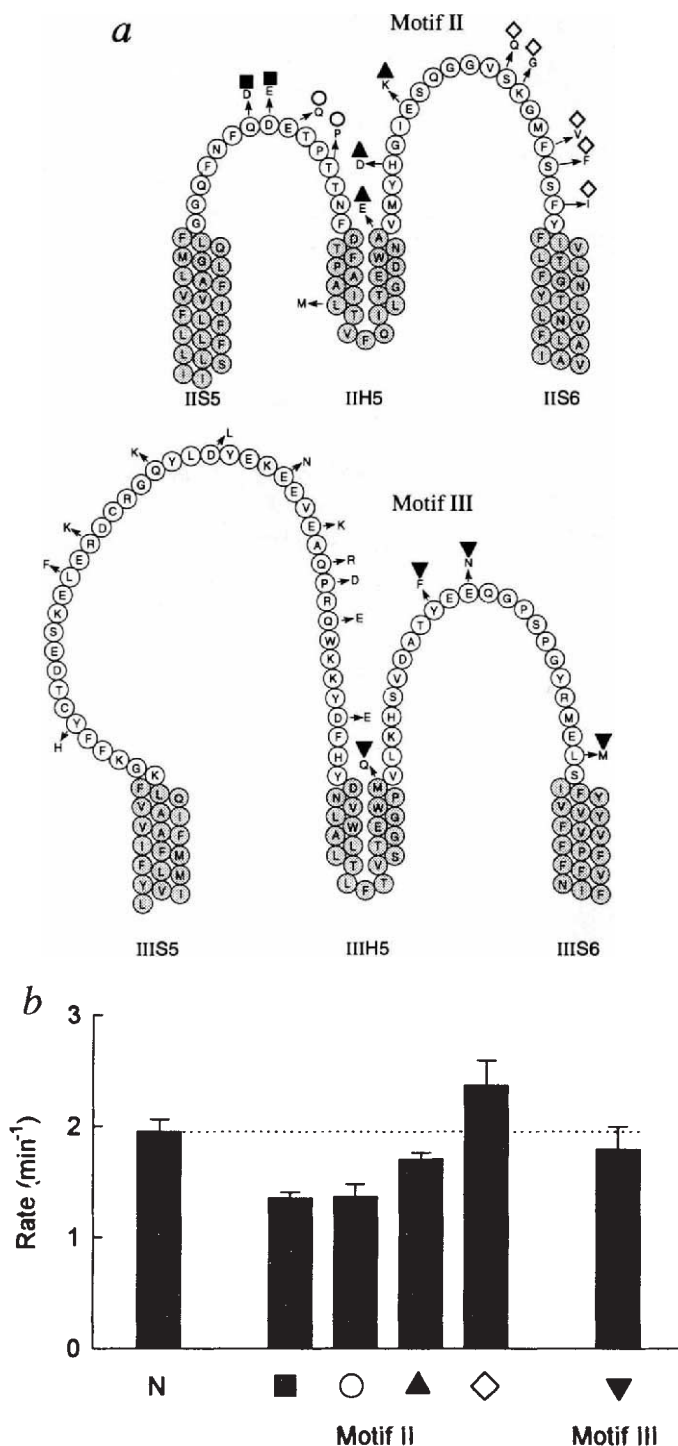


FIG. 3 Contributions of sets of amino acids within motifs II and III to the interaction between N-type Ca^{2+} channels and ω -CTx-GVIA. *a*, Schematic representation of the N-type channel between S5 and S6 in motifs II and III. Arrows signify positions where the sequence of α_{1A} differs from that in the N-type α_1 subunit (α_{1B}). Sets of amino-acid substitutions are indicated by different symbols. Shaded residues are putative transmembrane segments. *b*, Pooled values for the on-rate for toxin block for the normal N-type channel (N) and for the mutants in motifs II and III as indicated in *a*.

METHODS. Individual amino-acid changes were made using the megaprimer PCR method. Complementary DNA fragments were introduced into motif II at the *Bgl*III (1,462)–*Hind*III (2,339) sites of a modified version of the N-type channel which contained a deletion of the *Hind*III site in the polylinker, and into motif III at the two novel sites, *Mun*I (4,049) and *Sna*BI (4,331), engineered with the introduction of silent mutations.

variable finding was a partial reversibility of the block upon removal of ω -CTx-GVIA (Fig. 1d)^{5,24}.

Unlike N-type Ca^{2+} channels, Ca^{2+} channels encoded by α_{1A} are unresponsive to ω -CTx-GVIA (refs 22, 23; Fig. 2a). To identify structural determinants of susceptibility to ω -CTx-GVIA block, we constructed chimaeras comprising all possible combinations of motifs from toxin-sensitive (N) and toxin-insensitive (A) α_1 subunits (NA1–14; Fig. 2b). Although each of the motif chimaeras formed functional channels, the time course and degree of block by ω -CTx-GVIA varied widely. Chimaeras NA1–NA4 indicate how individual motifs contribute to the toxin-binding site, as only one motif is from A and the other three are from N. Subunit NA3 (NNAN) showed the greatest alteration in the onset of block relative to the parental N-type channel, a 9-fold slowing, whereas NA1 (ANNN) and NA2 (NANN) displayed a ~ 3 -fold decrease in on-rate, and NA4 (NNNA) showed only a ~ 1.3 -fold slowing. Another group of chimaeras (NA5–8) tested the impact of single motifs of the ω -CTx-GVIA-sensitive channel. Of these, only NA7 (AANA) was capable of conferring any detectable responsiveness to ω -CTx-GVIA ($\tau = 1.483 \pm 229$ s, $n = 4$) (Fig. 2a).

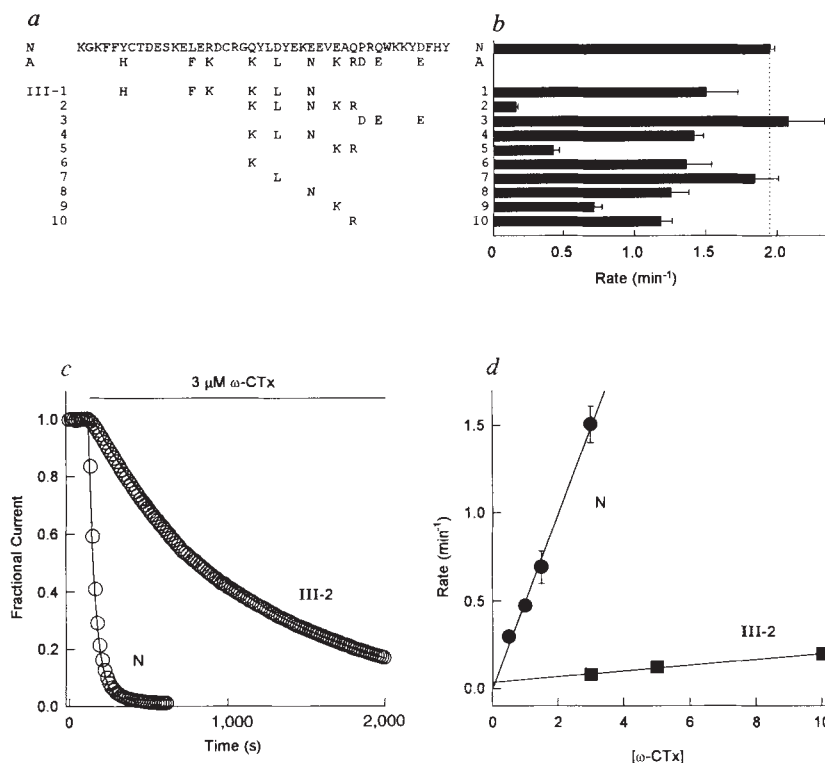
Chimaeras containing two motifs from each parent are grouped as complementary pairs, NA9/10, NA11/12, NA13/14 (Fig. 2*b*, right column). For the member of each pair that contains motif III, toxin block develops much more rapidly than for the one that does not. Indeed, block is undetectable for chimaera 9 (NNAA) or chimaera 12 (ANAN) and only very slow for chimaera 13 (NAAN). Thus, striking changes in toxin interactions result in every case where replacements were made in motif III, for N→A substitution against an N-type background, A→N substitution against a class A background, and with pairwise combinations of motifs in the N₂A₂ chimaeras.

ω -CTX-GVIA is known to act at the external face of the channel⁴, possibly by occluding the pore^{4,5} (but compare ref 18). Accordingly, we changed putative external loops flanking the pore-lining segment known as H5, switching sets of residues from those in the N-type channel to those in α_{1A} (Figs 3, 4). Figure 3*a* illustrates loop modifications in motifs II and III. In either motif, changes in the H5–S6 loop had little or no effect on blocking kinetics (Fig. 3*b*). In contrast, substitutions in IIS5–H5 (represented by filled squares and by circles in Fig. 3) caused appreciable slowing of the onset of block.

We made an extensive series of mutants (III-1 to 10) to analyse possible contributions of residues in the putative external loop between IIIS5 and IIH5 (Fig. 4a). At 9 of the 11 positions where N-type and α_1A channels differ, the respective amino-acid side chains bear a different charge. Among mutants III-1-3, which encompass all the amino-acid differences in the loop, only mutant III-2 displayed a significantly reduced rate of toxin block (Fig. 4b, c). The slowing caused by swapping five amino acids exceeded that produced by replacing motif III in entirety ($\tau = 370.4 \pm 34.2$ s, $n = 5$, versus $\tau = 273.8 \pm 29.9$ s, $n = 5$). Biophysical properties of the mutant III-2 channel were similar to the wild-type N-type channel (Fig. 4 legend). For both mutant III-2 and wild-type currents, blocking rate was linearly related to ω -CTx-GVIA concentration (Fig. 4d), consistent with one-to-one binding⁶. The slopes of the regression lines indicate association rate coefficients (k_{on}) of $0.49 \mu M^{-1} \min^{-1}$ (wild type) and $0.016 \mu M^{-1} \min^{-1}$ (mutant III-2), a 30-fold difference.

Effects of smaller subsets of the five amino-acid substitutions in mutant III-2 were tested (mutants III-4 to III-10). Replacements at the first three positions (mutant III-4) retarded block by 1.4-fold relative to wild type, whereas substitutions at the next two positions (mutant III-5; in single-letter code, amino acids E→K, Q→R) caused a 4.6-fold slowing. Replacement of single residues produced a slowing of 2.7-fold for E→K, and 1.6-fold for Q→R (III-9 to III-10). Indeed, four out of five amino-acid changes in mutant III-2 yielded clear effects even as

FIG. 4 The contribution of the IIIS5–IIIH5 region to ω -CTx-GVIA of N-type channels. **a**, The primary sequence of the N-type channel from IIIS5 to IIIH5 (first line), and specific differences in amino-acid sequence found in α_{1A} (second line). Shown below are mutants III-1 to III-10 of the N-type channel, containing one to six amino-acid replacements according to the sequence of α_{1A} . **b**, The on-rate for toxin block for the normal N-type channel and each of the mutants on the left. **c**, Time course of block by $3 \mu\text{M}$ ω -CTx-GVIA of the normal N-type channel (N) and mutant III-2. Smooth curves are single exponential fits (N, $\tau = 43.4$ s; and mutant III-2, $\tau = 994.6$ s). Biophysical properties of mutant III-2 were similar to wild type in all respects: significant activation at -20 mV in 5 mM Ba^{2+} , peak current between 0 and $+10$ mV, reversal potential of $+49.6 \pm 0.9$ mV ($n = 7$), midpoint voltage of steady-state inactivation $V_{1/2} = -66.8 \pm 1.2$ mV ($n = 10$). **d**, Concentration dependence of the rate of block (τ^{-1}). Pooled data were fitted with straight lines according to the relationship $\tau^{-1} = k_{\text{on}}[\text{toxin}] + k_{\text{off}}$. Slopes gave k_{on} values of $0.49 \mu\text{M}^{-1} \text{ min}^{-1}$ (wild type) and $0.016 \mu\text{M}^{-1} \text{ min}^{-1}$ (mutant III-2). Some levelling off of the on-rate for block was observed at higher doses, when the apparent rate was affected by the speed of bath solution change as gauged by the response to $3 \mu\text{M}$ Cd^{2+} (data not shown).



individual point mutations. The residues with significant impact on toxin interactions lie up to 45 amino acids away from a conserved glutamate involved in Ca^{2+} selectivity^{25,26}.

ω -CTx-MVIIA, another ω -conotoxin thought to act at the same site as ω -CTx-GVIA²⁷, produced a rapid and irreversible block of the N-type channel ($\tau = 17.3 \pm 0.7$ s, $n = 4$), and had no effect on α_{1A} ($n = 3$). With mutant III-2, toxin block developed with $\tau = 193.5 \pm 6.5$ s ($n = 6$), 11 times slower than for the parental N-type. Evidently, ω -CTx-MVIIA and ω -CTx-GVIA interact with at least some of the same channel constituents.

These experiments reveal molecular features of a voltage-gated Ca^{2+} channel that are important for its high-affinity interaction with a peptide toxin. The heterotetrameric architecture of the Ca^{2+} channels allowed us to examine the contributions of various motifs, singly and in combination. All four motifs are capable of influencing the interaction with toxin. Together, the various motifs interact more strongly with ω -CTx-GVIA than would be expected from the sum total of the individual behav-

iour of single motifs (Fig. 2c), in contrast to subunits of tetrameric *Shaker* K^{+} channels and the binding of charybdotoxin²⁸. The contributions of the motifs are unequal, motif III appearing the most important in conferring ω -CTx sensitivity. Together, N-type motifs I and III approach parental N-type behaviour more closely than any other pair. These motifs display larger and more variable putative external loops than motifs II and IV, and indeed, further mutagenesis within motif III established the importance of the large S5–H5 loop for ω -CTx sensitivity. These results support the idea that the ω -conotoxin molecule interacts with extracellular aspects of individual motifs, presumably by lodging within the outer vestibule of the channel³. As a large, rigid molecule of known three-dimensional structure, ω -CTx-GVIA may prove useful as a probe for charting the outer aspects of the Ca^{2+} channel (compare refs 9 and 29). In light of the close structural similarities among ω -conotoxins^{3,4}, conclusions for N-type channels and ω -CTx-GVIA may find general applicability. □

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GTPase mechanism of Gproteins from the 1.7-Å crystal structure of transducin α ·GDP·AlF₄⁻

John Sondek*, David G. Lambright*,
Joseph P. Noel*, Heidi E. Hamm†‡
& Paul B. Sigler*§

* Department of Molecular Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale University, 295 Congress Avenue, Boyer Center for Molecular Medicine, New Haven, Connecticut 06510, USA

† Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, Illinois 60680, USA

‡ Istituto di Fisiologia Generale e Chimica Biologica, Università di Sassari, Sassari 07100, Italy

§ To whom correspondence should be addressed

ALUMINIUM fluoride (AlF₄⁻) activates members of the heterotrimeric G-protein (G_{αβγ}) family^{1,2} by binding to inactive G_α·GDP near the site occupied by the γ -phosphate in G_α·GTP (ref. 3). Here we describe the crystal structure of transducin α ·GDP activated with aluminium fluoride (G_α·GDP·AlF₄⁻·H₂O) at 1.7 Å, a resolution sufficient to establish the coordination geometry of the bound aluminium fluoride as well as the extensive network of direct and water-mediated interactions that stabilize it. These observations are derived from three independent representations in the asymmetric unit, eliminating any chance of drawing conclusions based on stereochemistry imposed by crystal packing. Surprisingly, aluminium fluoride activates G_α·GDP by binding with a geometry resembling a pentavalent intermediate for GTP hydrolysis. The stabilizing interactions involve not only residues that interact with the γ -phosphate in G_α·GTP γ S, but also conserved residues essential for GTPase activity. Thus the

G_α·GDP·AlF₄⁻·H₂O structure provides new insight into the mechanism of GTP hydrolysis.

Figure 1a shows the conformation of the protein to be nearly identical to the active GTP γ S-bound form^{4,5}, except for local adjustments within segments that directly contact the bound aluminium fluoride. The aluminium ion occupies a site similar to the γ -phosphorus of active G_α·GTP γ S and is octahedrally coordinated with four fluoride ions in the equatorial plane and two oxygen atoms in the apical positions; one oxygen from the β -phosphate emulates the β - γ bridging oxygen of the triphosphate and the other oxygen is contributed by a water molecule (Fig. 2a). The most striking differences between the GDP·AlF₄⁻·H₂O and GTP γ S forms of G_α involve two residues, Thr 177 and Gln 200, which interact with and stabilize the aluminium oxyfluoride (Fig. 1b, c). In G_α·GTP γ S, the putative attacking water molecule is positioned by the peptide NH of Gln 200 and the peptide carbonyl of Thr 177. In G_α·GDP·AlF₄⁻·H₂O, a 180° flip of the peptide at Thr 178 coupled to the release of the peptide NH of Gln 200 allows the equivalent water molecule to move ~1 Å closer to the β -phosphate of GDP. This water molecule assumes an apical position in the GDP·AlF₄⁻·H₂O complex while maintaining a strong, almost ideal, hydrogen bond with the carbonyl of Thr 177. The side chain of Gln 200, held in a functionally neutral position through interaction with Lys 176 in G_α·GTP γ S, reorients to form two strong hydrogen bonds (~2.5 Å with nearly ideal geometry) to AlF₄⁻·H₂O, one between the amide NH and a fluoride ion and another between the carbonyl oxygen and the apical water. In order to maintain a water-mediated hydrogen bond, the carboxylate of Glu 39 also shifts, buttressing the side-chain amide of Gln 200 in its new position. The aluminium fluoride is further stabilized by interactions of the fluorides with the guanidinium group of Arg 174, the amino group of Lys 42, the backbone NH of residues 39, 177 and 199, and a Ca²⁺ ion (Fig. 2b). The accommodations made by the protein to stabilize aluminium fluoride involve residues that are essential for GTPase activity and are conserved in the heterotrimeric G-protein and p21^{ras} families. We suggest that these interactions

TABLE 1 Refinement statistics

Resolution shell (Å)	3.33	2.67	2.34	2.14	1.98	1.87	1.78	1.70	Total
Number of reflections	15,702	15,424	15,230	14,818	14,641	14,477	13,757	12,535	116,584
Per cent complete	97.2	94.5	91.6	88.4	87.1	86.5	82.3	76.7	88.0
$\langle I/\sigma \rangle$	25.3	22.8	19.2	16.7	13.5	9.62	6.43	4.22	20.6
R _{sym}	0.086	0.092	0.108	0.124	0.155	0.219	0.326	0.486	0.103
R-factor (all data)	0.177	0.200	0.230	0.247	0.275	0.326	0.404	0.524	0.235
R-factor (data >2.0σ)	0.174	0.196	0.218	0.228	0.240	0.258	0.276	0.298	0.209
Free R-factor (all data)	0.271	0.260	0.273	0.281	0.294	0.340	0.385	0.528	0.290
Free R-factor (data >2.0σ)	0.269	0.253	0.260	0.263	0.259	0.278	0.284	0.317	0.266
R.m.s. deviations	Bond lengths 0.007 Å				Bond angles 1.18°				

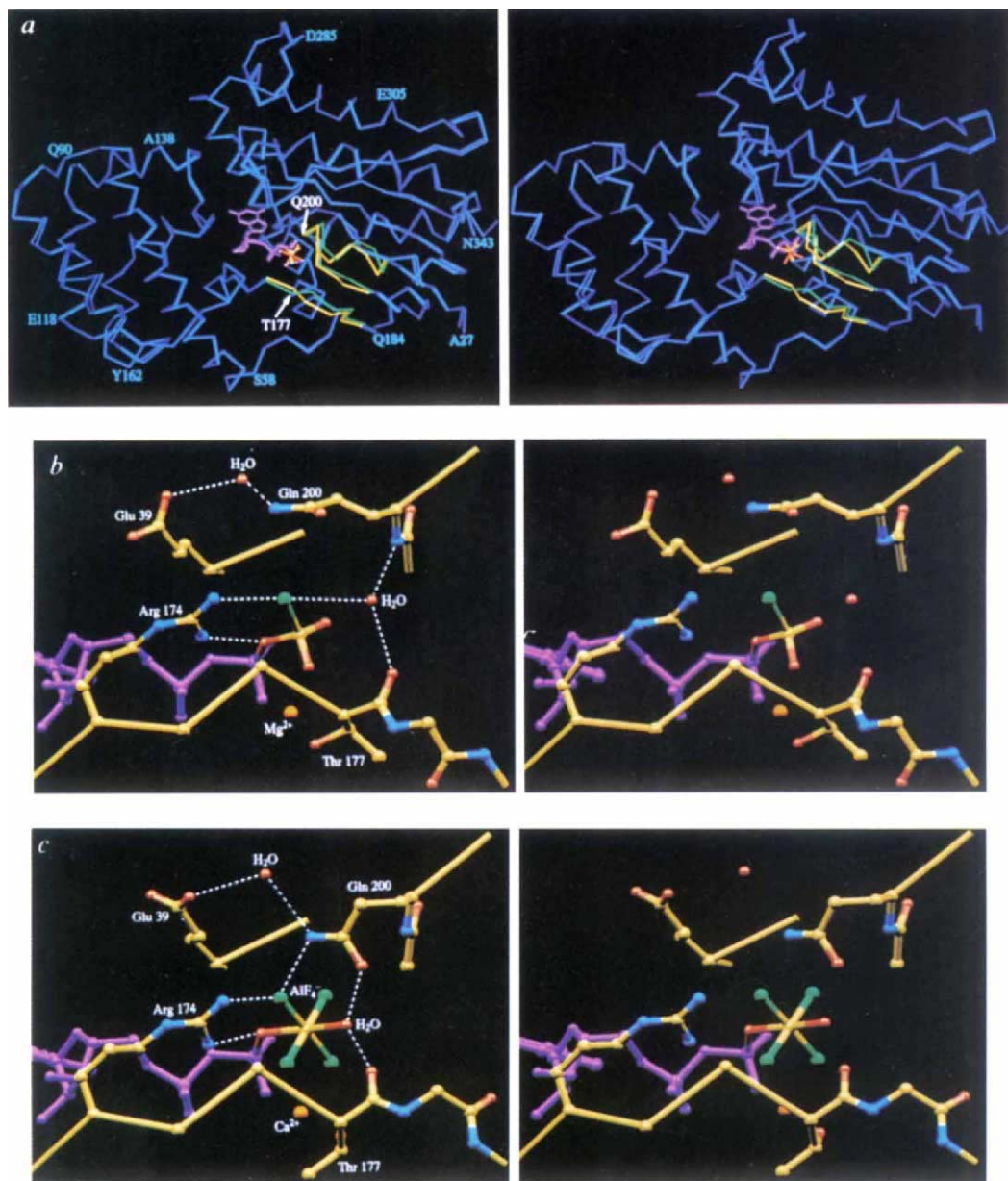
G_{αβγ} isolated from photolysed bovine retinal rod outer segments²⁶ was used to prepare a truncated form of G_α·GDP (residues 26–350) as described⁵. Crystals were grown in batch at 4 °C by the addition of 10 μl of a 20 mg ml⁻¹ solution of G_α·GDP to an equal volume of solution containing 20% w/v PEG-8000, 200 mM CaCl₂, 100 mM sodium cacodylate pH 6.0, 0.2% v/v β -mercaptoethanol, 5 mM MgCl₂, 100 μM AlCl₃, 30 mM NaF and 40% (v/v) glycerol. Diffraction quality crystal in the space group P2₁ grew within one week with cell dimensions of $a = 74.9$, $b = 108.2$, $c = 79.0$, $\alpha = 90$, $\beta = 111.7$, $\gamma = 90$. Crystals were stabilized in a cryosolvent containing the precipitating solution adjusted to contain 30% (v/v) glycerol and flash-frozen in liquid propane. Diffraction data were collected at the X-25 beamline of the National Synchrotron Light Source at Brookhaven National Laboratory, processed with DENZO (Z. Otwinowski), and scaled with SCALEPACK (Z. Otwinowski). The 2.2 Å refined crystal structure of G_α·GTP γ S (residues 27–340) was used as a starting model for rigid body minimization against data from 8–3 Å. The 3 protomers in the asymmetric unit were treated as independent rigid bodies. During independent refinement of the three protomers the resolution was extended to 1.7 Å in shells of 0.3 Å using simulated annealing and positional refinement. All refinement was done using XPLOR 3.1. Inspection of the difference electron density maps ($2|F_o| - |F_c|$ and $|F_o| - |F_c|$) at 1.7 Å showed clear density for the aluminium fluoride. Interactive model building was accomplished using the program O²⁷. The current model has three protomers (A–C) containing amino acids corresponding to 27–342, 27–342 and 27–343 of the full-length sequence. The model also contains 3 molecules of: GDP, Ca²⁺, AlF₄⁻·H₂O, 1,010 waters having good hydrogen bonding geometry and B-factors less than 60 Å², as well as previously identified Cys 62 and Cys 210 modified with arsenate. For all but one round of refinement, reasonable restraints were applied to: Al–F bonds (1.80 Å), Al–H₂O bonds (2.00 Å), F–Al–F angles (90°) and H₂O–Al–F angles (90°). The high CaCl₂ in the crystallization and stabilizing conditions plus the unrealistically low thermal B-factors when Mg²⁺ was used in the model suggested that Ca²⁺ may have replaced Mg²⁺ under these conditions. Occupancies and B-factors in subsequent refinement were also consistent with the divalent cation being Ca²⁺. All graphics presented used the programs O and Ribbons²⁸. $R_{\text{sym}} = \sum_h \langle |I_h - \bar{I}_h| \rangle / \sum_h I_h$, where $\langle |I_h - \bar{I}_h| \rangle$ is the average of the absolute deviation of a reflection I_h from the average $\bar{I}_h$ of its symmetry and Friedel equivalents.

or stereochemically similar ones serve a general role in promoting and stabilizing the pentavalent intermediate of GTP hydrolysis as described later.

As water attacks a γ -phosphate, it must be deprotonated. However, negatively charged, high-energy phosphoanhydrides are kinetically stable to hydrolysis because they repel nucleophilic attack by negatively charged hydroxide ions⁶. On the basis of the $G_{ta} \cdot GTP\gamma S$ and $G_{ta} \cdot GDP \cdot AlF_4^- \cdot H_2O$ structures (Fig. 3a) we consider two potential mechanisms for the production and stabilization of the pentavalent intermediate which would both deprotonate the attacking water molecule and overcome the electrostatic barrier by reducing the negative charge on the

phosphate. Figure 3b shows a concerted mechanism in which the side chain of Gln 200 abstracts a proton from the attacking water molecule while simultaneously donating a proton to a γ -phosphate oxygen in a manner reminiscent of other tautomer-based catalytic mechanisms⁷⁻⁹. The pentavalent intermediate would be stabilized by hydrogen bonds to the imino tautomer of Gln 200, with an orientation identical to that in $G_{ta} \cdot GDP \cdot AlF_4^- \cdot H_2O$. Alternatively (Fig. 3c), the attacking water molecule could transfer a proton directly to the γ -phosphate. This is plausible because a water molecule in $G_{ta} \cdot GTP\gamma S$ (ref. 4) and another in the GppNp-bound form of p21^{ras} (ref. 10) are both positioned with almost ideal geometry for direct

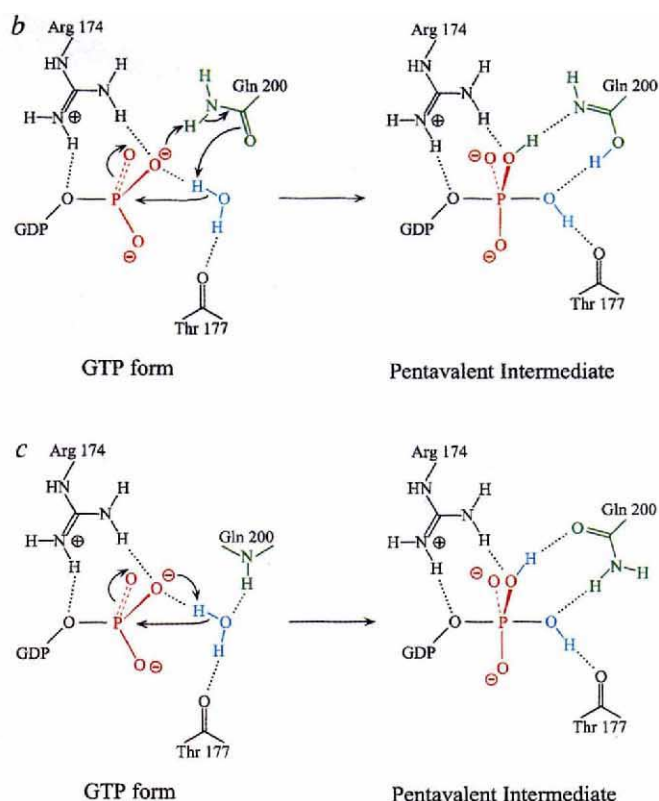
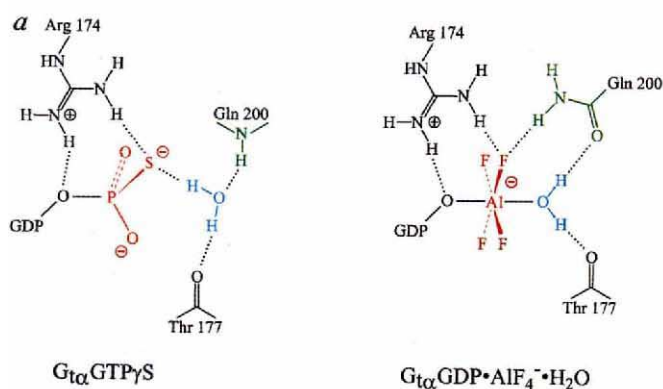
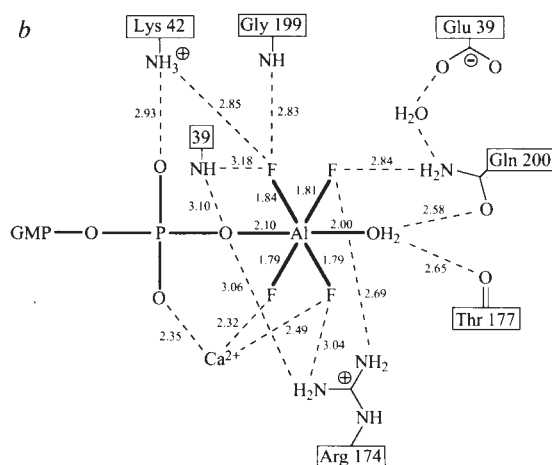
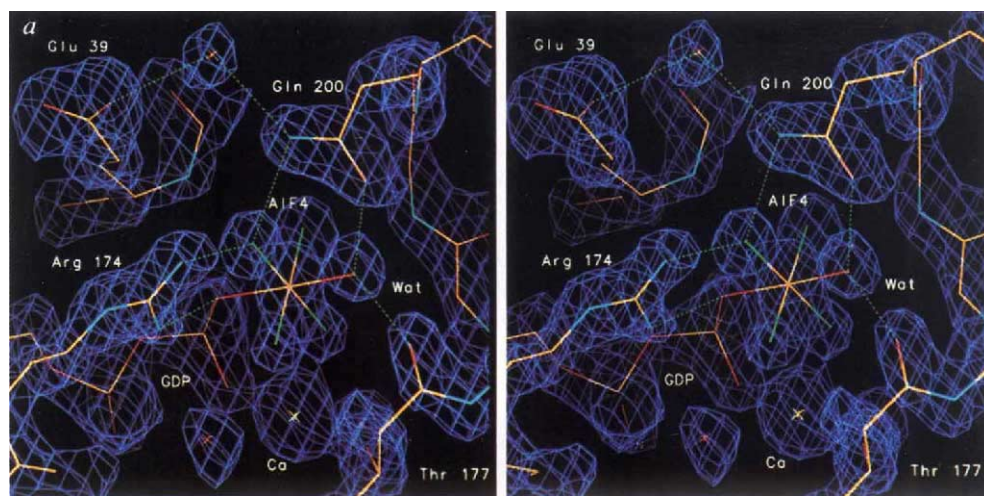
FIG. 1 Stereoviews comparing G_{ta} bound with $GTP\gamma S$ or $GDP \cdot AlF_4^- \cdot H_2O$. a, Least-squares superposition of α atoms. Common structure is green, segments that differ (176–182 and 195–207) are yellow for $G_{ta} \cdot GTP\gamma S$ and blue for $G_{ta} \cdot GDP \cdot AlF_4^- \cdot H_2O$. Nucleotides are purple except the γ -thiophosphate in yellow and $AlF_4^- \cdot H_2O$ in red. Corresponding representations in the asymmetric unit have an average r.m.s. difference of $0.403 \pm 0.003 \text{ \AA}$ for α atoms and $1.11 \pm 0.011 \text{ \AA}$ for all atoms. b, Stereoview of the γ -phosphate region of $G_{ta} \cdot GTP\gamma S$, emphasizing the interactions of the putative attacking water molecule (peptide NH of Gln 200 and the carbonyl of Thr 177, sulphur (green) of $GTP\gamma S$). Carbons are yellow, oxygens red, nitrogens blue and the nucleotide is purple. Mg^{2+} is an orange sphere and dotted lines represent hydrogen bonds. For clarity, the main chain is a α -trace except for 177–178, and only side chains that stabilize the γ -phosphate and the putative attacking water are shown. c, Stereoview of the active site of $G_{ta} \cdot GDP \cdot AlF_4^- \cdot H_2O$ orientated and colour coded as in b, except that green represents fluoride. The hydrogen bond to the amide backbone of Gln 200 is broken and the side chain of Gln 200, buttressed by Glu 39, reorients to form strong hydrogen bonds with the apical water molecule and a fluoride ion. The carbonyl of Thr 177 reorients to maintain a hydrogen bond with the apical water, and the main chain following 177 reorients. Glutamate 203, which maintains a similar interaction with Lys 206 in both structures, is probably not directly involved in the catalytic mechanism as previously suggested⁴. The divalent cation (Ca^{2+} appears to replace Mg^{2+} during crystallization) coordinates 5 ligands identified in $G_{ta} \cdot GTP\gamma S$ (ref. 4) and replaces an oxygen of the γ -phosphate with two equatorial fluoride ions giving Ca^{2+} a total of 7 ligands. If the complex of $G_{ta} \cdot GDP \cdot AlF_4^- \cdot H_2O$ were an analogue of the pentavalent intermediate for GTP hydrolysis, we would expect that in addition to trigonal bipyramidal geometry around the



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γ -phosphorus, a pentavalent intermediate for GTP hydrolysis would have: (1) increased negative charge on the γ -phosphate oxygens; (2) increased P-O bond lengths; and (3) an attacking nucleophilic water or hydroxide ion in an apical position. Whereas $GDP \cdot AlF_4^- \cdot H_2O$ has octahedral geometry around the Al^{3+} with four fluoride ions in the equatorial plane instead of three oxygen atoms, it also has: (1) substantial negative charge localized to the fluoride ions, (2) Al-F bonds that are longer than the P-O bonds of GTP; and (3) an apical water occupying the 'in-line' position of an attacking nucleophile.

FIG. 2 Stereochemistry of interactions between G_{α} and $GDP \cdot AlF_4 \cdot H_2O$. **a**, Stereoview at 1.7 Å of a $(2|F_o| - |F_c|)$ annealed omit map of the electron density centred on the aluminium fluoride complex and contoured at 1.4σ . To avoid a biased image, the portions of the model displayed were omitted before a round of simulated annealing refinement and phase calculation. The density is representative of all three molecules in the asymmetric unit and indicates the resolution and quality of the structure with which we determined the nature and geometry of the aluminium fluoride complex. Atom colours are those described in Fig. 1b,c. **b**, Illustration of the interactions between $GDP \cdot AlF_4 \cdot H_2O$ and stabilizing residues. All interactions involve highly conserved residues within the GTPase superfamily and are observed in each of the three molecules of the asymmetric unit. During one round of refinement, the twelve Al-F bonds, the three Al to water-oxygen bonds, and the three Al to phosphate-oxygen bonds refined without restraints to average values of 1.73 ± 0.07 , 1.95 ± 0.06 and 1.98 ± 0.08 Å, respectively. The agreement with the bond distances for Al-F (1.80 Å) and Al-O (2.00 Å) observed in small molecule crystal structures^{29,30} identifies the bound species as $GDP \cdot AlF_4 \cdot H_2O$ and attests to the accuracy of the model. The strong hydrogen bond observed between the carbonyl oxygen of Thr 177 and the apical ligand rules out fluoride ion at the apical position. The angle of 109° formed between the side-chain oxygen of Gln 200, the apical water, and the carbonyl oxygen of residue 177 is ideal for hydrogen bonds to water. Hydrogen bonds between the oxygen of the apical water and the side chain oxygen of Gln 200 average 2.56 ± 0.015 ; between the oxygen of the apical water and the carbonyl oxygen of Thr 177 they average 2.64 ± 0.032 . The restrained bond lengths in the illustration are for one of the complexes.



proton transfer. In this case, the pentavalent intermediate would be stabilized by hydrogen bonds to the side-chain amide of Gln 200, with an orientation opposite to that in $G_{1\alpha} \cdot \text{GDP} \cdot \text{AlF}_4^- \cdot \text{H}_2\text{O}$. In both mechanisms, the γ -phosphate is the ultimate general base, as proposed for $p21^{\text{ras}}$ (refs 11 and 12, and A. Wittinghofer, personal communication). Arg 174 facilitates production of the leaving group⁴, and Gln 200 stabilizes the pentavalent intermediate as proposed for $p21^{\text{ras}}$ (ref. 13) and subsequently for $G_{1\alpha 1}$ (ref. 14). Finally, many of the critical interactions involved in the catalytic models presented here are facilitated by rearrangements within regions known to affect GTPase-activating protein (GAP) binding and activity^{15–18}. We speculate that GAPs accelerate GTPase rates by reorienting these regions in order to stabilize the pentavalent intermediate in a manner similar to that described here.

Mutational analysis is consistent with these mechanisms. Substitution of Gln 61 in $p21^{\text{ras}}$ (Gln 200 in $G_{1\alpha}$) is a frequent mutation¹⁹, and 17 different substitutions of Gln 61 lead to a similar ~10-fold reduction in the intrinsic GTPase rate²⁰. Substitution of the homologous Gln 227 in G_{sa} also reduces the intrinsic rate of GTP hydrolysis and is associated with thyroid²¹ and pituitary tumours²². Substitution of Gln 61 with either a glutamic acid residue¹² or an unnatural amino acid containing a nitro group²³ in place of the amide has little effect on the intrinsic or GAP-stimulated hydrolysis rate. These groups could partially substitute for the amide by hydrogen-bonding to a protonated phosphate. Substitutions of Gly 12 in $p21^{\text{ras}}$ (Gly 38 in $G_{1\alpha}$), conserved throughout the GTPase superfamily and the site of the most frequent oncogenic mutation in $p21^{\text{ras}}$ (ref. 19), also disrupt intrinsic GTPase activity. Like the GTP-bound forms of $p21^{\text{ras}}$ and $G_{1\alpha}$, Gly 38 in $G_{1\alpha} \cdot \text{GDP} \cdot \text{AlF}_4^- \cdot \text{H}_2\text{O}$ does not have unusual ϕ - ψ angles. However, the structure of $G_{1\alpha} \cdot \text{GDP} \cdot \text{AlF}_4^- \cdot \text{H}_2\text{O}$ shows that a β -substituent would weaken the stabilization of the pentavalent intermediate by sterically hindering the interaction of Gln 200 with a protonated, equatorial phosphate oxygen as well as the water-mediated hydrogen bond between Gln 200 and Glu 39. Other residues described as interacting with $\text{GDP} \cdot \text{AlF}_4^- \cdot \text{H}_2\text{O}$ (Gly 199 for example) are also conserved in G-protein families and affect GTPase rates²³. In general, the ability of aluminium fluoride to affect numerous enzymes^{24,25} that catalyse the hydrolysis of nucleotides, including tubulin, myosin, F₁-ATPase, hexokinase, and actin suggests that our conclusions may be applicable to many other systems. □

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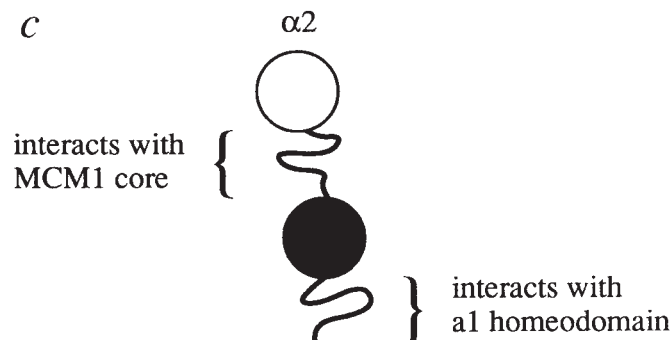
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ERRATUM

Interaction between two homeodomain proteins is specified by a short C-terminal tail

Martha R. Stark & Alexander D. Johnson

Nature **371**, 429–432 (1994)



PART c of Fig. 1 of this Letter was accidentally omitted and is now shown here. □

◀ FIG. 3 Stereochemistry of the GTPase mechanism. a, Interactions involving $\text{GTP}\gamma\text{S}$ or $\text{GDP} \cdot \text{AlF}_4^- \cdot \text{H}_2\text{O}$ at the active site of $G_{1\alpha}$ immediately relevant to the GTPase mechanism. $\text{GDP} \cdot \text{AlF}_4^- \cdot \text{H}_2\text{O}$ mimics the pentavalent intermediate; that is, the square planar arrangement of fluoride ions coordinating the Al^{3+} ion is replaced by a triangular planar arrangement of oxygen atoms surrounding the γ -phosphorus. The positively charged guanidinium group of Arg 174 would increase the transfer of negative charge from the attacking hydroxyl to the β - γ bridging oxygen, facilitating the collapse of the pentavalent intermediate to a terminal β -phosphate and a free orthophosphate. Omitted for clarity are supporting ligands found in similar conformations in both $G_{1\alpha} \cdot \text{GTP}\gamma\text{S}$ and $G_{1\alpha} \cdot \text{GDP} \cdot \text{AlF}_4^- \cdot \text{H}_2\text{O}$. b, Concerted mechanism for GTP hydrolysis in which transfer of a proton to the γ -phosphate is coupled to the deprotonation of the attacking water. Tautomerization of Gln 200 enhances the nucleophilicity of the attacking water, reduces the negative charge of the γ -phosphate and stabilizes the pentavalent intermediate through two hydrogen bonds. c, Alternative mechanism for GTP hydrolysis involving direct transfer of a proton from the attacking water molecule to the γ -phosphate oxygen. This transfer is aided by the peptide NH of Gln 200 which is polarized by its contact with Arg 204. Here the pentavalent intermediate is stabilized by hydrogen bonds to a reorientated Gln 200 side-chain amide.

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Behind the atomic curtain

Hans A. Bethe

Stalin and the Bomb: The Soviet Union and Atomic Energy, 1939–1956. By David Holloway. Yale University Press: 1994. Pp. 464. \$30, £19.95.

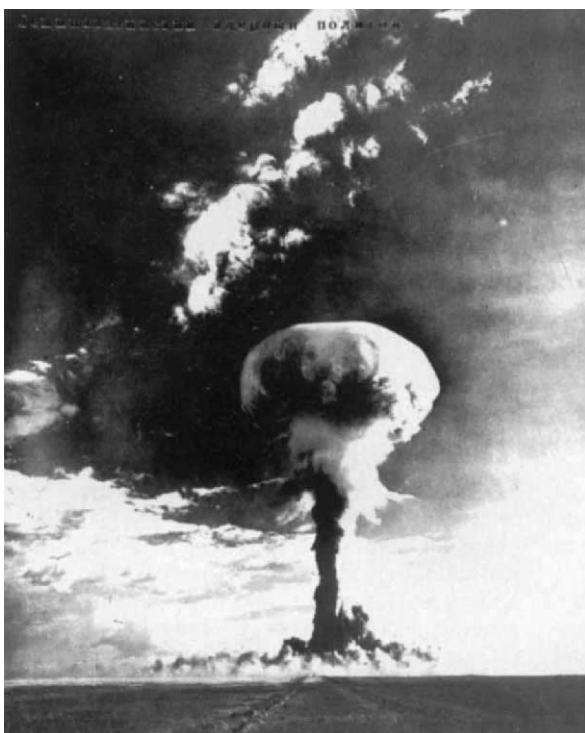
In 1945 we speculated that it would take the Russians about five years to develop an atomic bomb. The Soviet test came in August 1949, just about on time, but we did not know how they did it.

David Holloway found out. He made many trips to Russia, reading the now-available reports from the project that were compiled during and after the Second World War, and talking to many Russian scientists. He has produced a monumental work, with more than 2,000 references and notes. It makes fascinating reading and will remain the standard work on the subject for many years. What makes it particularly intriguing is that it clears up many previously unanswered questions. Many people have wondered, for instance, whether the nuclear arms race could have been avoided if the Americans or the British, or both, had acted appropriately. By studying Stalin's thinking, Holloway shows that such avoidance would have been impossible.

The centre for physics research in Russia in the 1930s was Ioffe's Institute in Leningrad. Igor Kurchatov, who would head the Soviet bomb effort, joined the institute in the early 1930s, where he worked in nuclear physics. In 1934, impressed by Enrico Fermi's work with neutrons, he specialized in neutron physics. The discovery of fission in 1938 caused great excitement among Russian scientists; Georgii Flerov, one of Kurchatov's collaborators, was the first to observe spontaneous fission, whereas Yulii Khariton and Yakov Zel'dovich developed the theory of the chain reaction. However, a problem for the Russian researchers was that very little uranium was available.

The Germans invaded the Soviet Union in June 1941. The Ioffe Institute was moved to Kazan; most of its members were recruited for war work. Fission work was discontinued. The British government, however, was taking the chain reaction most seriously, and appointed the Maud committee to analyse the situation. The committee, which included Britain's leading nuclear scientists, reported in July 1941 and recommended a determined effort to use the uranium chain reaction. The difficult isotope separation was to be accomplished by thermal diffusion. Spies

reported the conclusions of the Maud report to Russia around September 1941. In March 1942 Lavrentii Beria, the Soviet secret-police chief, told Stalin of the findings. Long consultations with scientists followed. Kurchatov was appointed to head a chain-reaction project; he was to report to Mikhail Pervukhin. The go-ahead came from Stalin in January 1943, after the battle of Stalingrad had turned in favour of the Soviets.



The Soviet hydrogen bomb test of 12 August 1953. This bomb was developed by the Soviets entirely independently, without espionage.

In March 1943 Kurchatov was shown the intelligence reports from Britain. On 7 March he reported to Pervukhin that a strategy similar to the British one was exactly what was needed. The British hoped that a slow-neutron chain reaction could be obtained with natural uranium in graphite, and Fermi was working on this task in Chicago. There was no mention that he had already succeeded on 2 December 1942, and Kurchatov asked intelligence urgently to find out the status of Fermi's work. The British report mentioned the use of plutonium. In 1943 Klaus Fuchs reported from Britain details about isotope separation. Fuchs went to the United States in December 1943. The espionage in

Britain was vital to the Soviet scientists.

Kurchatov was not allowed to tell his colleagues about the British information. With them, however, he planned a chain-reaction programme. He took charge of the experimental 'pile' of uranium. He decided to use graphite; 50–100 tons of uranium would be needed to make the pile go critical. An exponential pile was designed by January 1944, and a cyclotron was brought from Leningrad and assembled by September 1944. By irradiating uranium, the first plutonium was produced. Kurchatov requested 100 kg of uranium oxide and metal from Lend Lease in Washington; the request was approved by General Leslie Groves, the head of the Manhattan Project, and the materials delivered in 1943. Later Kurchatov acquired about 300 kg from occupied

Germany. Although these were minuscule amounts for building a reactor, they were vital for experimentation.

Theoretical work was started under the leadership of Khariton. Fuchs, now at Los Alamos, provided basic data about the spontaneous fission of plutonium-240, the need for implosion to assemble the plutonium bomb and the need for explosive lenses. In June 1945 he fully described the plutonium bomb, including a sketch and all important dimensions. He wrote that the expected energy yield was 10 kilotons. Kurchatov reported that the data from Fuchs had enormous significance.

As the Soviet army advanced into central Europe, the Czech president, Edvard Beneš, signed an agreement allowing Soviets to mine uranium in Czechoslovakia. Several important German scientists joined the Russian project.

In July 1945, at the Potsdam Conference, President Harry Truman told Stalin that the United States had a weapon of unprecedented power that it would use against Japan. On 6 August the US

Air Force dropped an atomic bomb on Hiroshima. On 8 August the Soviet Union attacked Japan's Manchurian army, defeating it easily. Stalin had promised to enter the war against Japan at about this time, but now the Soviet involvement embarrassed the United States.

After Hiroshima, Stalin immediately ordered an all-out effort in the building of Russia's atomic bomb. He put Beria in charge of the project, and told Kurchatov that cost was no object. Holloway makes it clear that Stalin would not have changed his attitude if the United States had followed the advice that Niels Bohr had given in 1944 and had told the Soviets earlier about the existence of the US – British project.

Nor was Stalin's mind altered by the Acheson-Lilienthal plan to internationalize all nuclear developments. This plan was presented to the United Nations by the US statesman Bernard Baruch, who added some features that made it less acceptable to the Soviets. But even without these features, the Soviets would have rejected the plan: Stalin simply wanted his own bomb.

Stalin and Beria were suspicious of almost everyone. They distrusted their own scientists in the atomic project, including Kurchatov; their own intelligence service and spies such as Fuchs; and the official US account of the uranium project, the Smyth report. After all, they asked, why should a country such as the United States publish all the steps leading to an atomic bomb?

Because of these suspicions, Beria sent Yakov Terletsii, a physicist and a member of the NKVD (Soviet police and secret police) to meet Bohr. Bohr, who announced the visit to both the Danish and the British governments, did not tell Terletsii anything beyond what was in the Smyth report, and Terletsii considered the trip to be a failure. But Beria thought it was a success, and in a letter to Stalin (recently published) he enumerates some technical points in the Smyth report, presenting them as if they had come from Bohr.

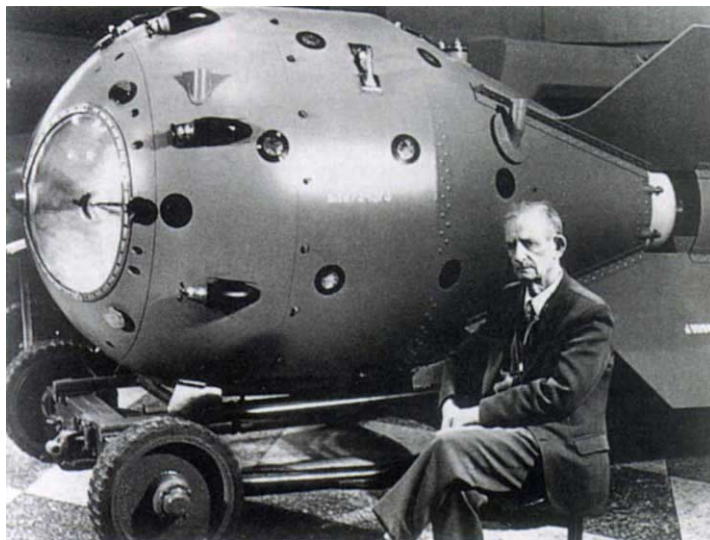
The Soviet uranium project was a remarkable technical achievement. Industry in the European part of Russia had been largely destroyed during the Second World War, but the industrial plants for plutonium production were built up in record time. Pure graphite was obtained by the end of 1945, uranium metal by the summer of 1946. The experimental reactor went critical on 25 December 1946. Kurchatov himself directed the approach to criticality, just as Fermi had done in 1942. Beria was not impressed; he still suspected that Kurchatov was deceiving him.

Assembly of the production reactor was started in March 1948; the reactor went critical in June 1948. Chemical separation of plutonium was achieved by precipitation. The project employed 20,000 to 30,000 people in production, 50,000 to 60,000 in construction and about 350,000 in mining. Mining and construction were done largely by prisoners. To prepare for the actual assembly of a bomb, a new laboratory was created, 60 km south of Arzamas; it was named Arzamas-16, and directed by Khariton.

Khariton and Zel'dovich repeated the

US calculations. Others in the laboratory repeated the experiments on implosion, a difficult task because Soviet electronics was far behind the Americans'. Explosive lenses were made — the Institute of Chemical Physics had much experience with explosives. The difficult problem of detonating all lenses simultaneously was solved to the same accuracy as in the United States.

There was the strictest secrecy in Arzamas. Reports were often written by hand and code words were used. Arzamas naturally was dubbed Los Arzamas. There was



Khariton with a replica of the bomb detonated on 29 August 1949.

a spirit of cooperation similar to that at Los Alamos. All scientists believed that the Russian bomb was necessary.

It took four years to build the bomb, just about the same as the US project. The United States had the advantage of being a fully functioning country, whereas Russia was largely destroyed. On the other hand, the Soviets had information from Fuchs that allowed them to bypass many difficulties. Fuchs thought he had saved the Soviets four to five years of work; it was more likely two to three.

Enough plutonium was ready before uranium-235, and the Russians had a complete design of the US plutonium explosive from Fuchs, so plutonium was chosen for the first test. The test took place on 29 August 1949 at Semipalatinsk, with Kurchatov in command. It was a complete success. Kurchatov wrote the report by hand. Receiving it, Stalin said: "There will not be war." Big rewards went to the scientific leaders, the most important of whom were declared "heroes of socialist labour", the highest distinction.

The Soviet bomb project had one very good side-effect. Tofim Lysenko had destroyed Soviet genetics. He and others now wanted to make physics also conform to Soviet ideology. Relativity theory and quantum theory were to be declared counter-revolutionary. A big meeting to

do this was scheduled for 21 March 1949. Fortunately, Kurchatov was consulted and said: "If quantum theory and relativity are eliminated, there cannot be any atomic bomb." Stalin accepted Kurchatov's opinion and cancelled the conference, five days before it was to begin. "We can always shoot them later", he said.

The hydrogen bomb was developed by the Soviets entirely independently, without espionage. The main inventor was Andrei Sakharov. His first idea was to use a "layer cake" with alternating layers of uranium-235 and deuterium. Vitalii

Ginzberg had the second idea, namely to supply deuterium in the form of solid lithium deuteride. A device using these two ideas was tested in August 1953 and gave a yield of 400 kilotons. Then Sakharov had a third idea, radiation implosion, the same as the US principle originated by Teller and Ulam. This was tested on 22 November 1955, with a bomb dropped from an airplane. It gave a yield of 1.6 megatons, the yield being kept low to permit the plane to escape undamaged. According to Sakharov's memoirs, the test "essentially solved the problem of high-performance thermonuclear weapons".

The Soviet test came 20 months after the US test of deliverable hydrogen bombs. The Soviets got no help from the US test of November 1952; although they had collected fallout in the snow, the sample was inadvertently poured down the drain by a chemist.

The General Advisory Committee to the US Atomic Energy Commission had tried in 1949 to prevent the hydrogen bomb by proposing to the Russians a treaty pledging both sides to refrain from its development. Sakharov and Khariton both believe that Stalin would never have accepted this.

After Hiroshima, President Truman and Secretary of State James Byrnes tried atomic diplomacy, but it was a failure. Stalin refused to be impressed; in fact, he pursued a confrontational policy. In the end, his policy led to the 1947 Marshall Plan for the postwar reconstruction of Europe.

After Stalin's death in March 1953, his successors remained committed to nuclear weapons. For instance, they declared surface ships obsolete in nuclear war. Like the Americans, the Soviets developed tactical weapons and instructed the military about nuclear war. Stalin's successors nevertheless pursued the idea of "peaceful coexistence" with the capitalists. At the Twentieth Party Congress in

From Stalin and the Bomb

January 1956, Khrushchev declared that there was a choice between peaceful co-existence and the most destructive war in history.

Khrushchev was impressed by the 1955 test of the improved hydrogen bomb. East and West now shared an understanding that nuclear war was unacceptable, and they knew that the other side understood this too. At last Bohr's dream was realized that statesmen should appreciate that nuclear weapons are a mortal danger to the world and are not weapons of war.

I can give here only a bare outline of Holloway's book. There is a lot more in it. For instance, he gives a detailed, vivid account of the Soviet-US confrontation in atomic policy, how it led to the Marshall Plan, to the establishment of the Federal Republic of Germany and its currency and to the Berlin blockade and airlift — all of it based on extensive documentation. Holloway is also good on the Korean War and the related discussions in the Soviet Union, and tells of the fate of well-known Soviet scientists.

This is a must read for all who are interested in the influence of atomic weapons policy, the early Cold War or the interplay of technical competence and espionage, as well as those simply looking for a splendid detective story. □

Hans A. Bethe is in the Newman Laboratory of Nuclear Studies, Cornell University, Ithaca, New York 14853-5001, USA. During the Second World War he was director of the theoretical physics division of the Los Alamos Atomic Scientific Laboratory.

Best of British

F. H. Hinsley

Test of Greatness: Britain's Struggle for the Atom Bomb. By Brian Cathcart. John Murray: 1994. Pp. 301. £19.99.

THE first British nuclear bomb was successfully exploded on 3 October 1952 in the Monte Bello islands off the northwest coast of Australia. This was the outcome of a programme — Brian Cathcart rightly calls it a struggle — that was propelled by a series of decisions stretching back to the second half of 1945. The decisions, which were so secret that the prime minister, Clement Attlee, did not consult or even inform his cabinet except for a few leading ministers (the Australian prime minister, Robert Menzies, followed suit), were prompted by the unilateral termination by the United States of the cooperation with Britain in the Manhattan Project which had produced the bombs dropped on Hiroshima and Nagasaki.

There were other considerations. A research programme seemed necessary if

Britain was to participate in the establishment of some system of international control of atomic energy, although the prospects of success were judged to be faint. Far stronger was the expectation that the Soviet Union would soon produce a bomb: the defector Igor Gouzenko disclosed in September 1945 that Alan Nunn May had passed information about the Manhattan Project to Moscow. Together with the realization that, as Attlee wrote within days of the explosions over Japan, the bomb had made nonsense of all previous thinking about the defence of Britain, these were the arguments that led to a rapid if not quite complete consensus behind the conclusion voiced in 1951 by William Penney, head of the team of scientists who designed the British bomb: "the discriminative test for a first-class power is whether it has made an atomic bomb". Ernest Bevin, the foreign secretary, is said to have made the point more colourfully in October 1946 to a cabinet committee dismayed at the estimated cost: "We've got to have this thing... whatever it costs.... We've got to have a bloody Union Jack flying on top of it."

A comprehensive account of these central political and strategic decisions, as also of the organizational, technological, financial and manpower problems to which they gave rise, and of how they were solved, was published in 1974 in the second volume of Margaret Gowing's official history, *Independence and Deterrence: Britain and Atomic Energy, 1945-52*. Cathcart provides a lucid summary of the official account and occasionally amplifies it where newly declassified documents allow. But decisions and developments at the higher levels, while they form the essential scaffolding for his book, are not his main concern. That is to relate how the project was brought to fruition at the workplace, his prime sources the recollections of a wide cross-section of the scientists assembled by Penney to staff High Explosive Research, forerunner of the Atomic Weapons Research Establishment at Aldermaston, and of the representatives from the Royal Air Force, the Royal Navy and the Royal Engineers who were directly associated with them; and as these men have recently become free to discuss their experiences — although still within certain security constraints — the result is an original and absorbing contribution to our knowledge.

The team included none of the country's foremost scientists. Penney set his sights on making a near approximation to the plutonium implosion bomb used against Nagasaki, of which he had acquired general knowledge at Los Alamos and as an observer of its effects from the air and on the ground. For this he needed not a reconstitution of the British Los Alamos team, but technologists and technicians. More than half of them were

in their 20s. They were, however, forced to work at and beyond the frontiers of their technological experience in chemistry and chemical engineering, nuclear physics, radiology, medicine and above all metallurgy. The refusal of the US authorities to provide information and material was offset by assistance from some of the British Los Alamos veterans. Notable among them was Klaus Fuchs; he had close knowledge of the Nagasaki weapon and, now head of theoretical physics at the Atomic Energy Research Establishment at Harwell, he placed this knowledge at Penney's disposal until in January 1950 he confessed that he had also assisted the Soviet Union, whose first nuclear test had recently been detected, and was jailed for treason.

Cathcart is not himself a scientist, and he has taken pains to make his account accessible, as literary critics say, to readers who labour under the same disadvantage. If the book has a fault it is that, between the fascinating story of the making of the bomb and the final drama of the test itself, it covers in equally great detail other matters, such as the transfer of the scientists and their equipment to Monte Bello and the preparations and rehearsals on the islands for the explosion, of which day-to-day and sometimes hour-to-hour accounts are bound to become tedious. But even this is a fault in the right direction: it completes the story and enhances its realism.

Eighty-five of the scientists and most of the equipment made the passage in cramped warships, and the voyage round the Cape took 59 days, 37 of them at sea. The Monte Bello islands held few species of wildlife and even fewer charms; and it causes no surprise to learn that hitches were frequent in the final preparations for so complex an undertaking in such hostile terrain. The truly serious hitch, not unlike that which had delayed and threatened to abort the Normandy landings in 1944, came at the end. If a lull in the unstable weather had not permitted a postponed explosion on 3 October, Cathcart doubts whether the expedition could have waited for the next suitable period.

He ends on an elegiac note. The success of the test marked only the entry of Britain into a race of indefinite duration — a race in which it was able to surmount the hurdle of the hydrogen weapon, a device first exploded by the United States in November 1952, but was thereafter outpaced by the development of ever more sophisticated delivery systems. He has no doubt, however, that Britain's decision to join in the race was justified, and he demonstrates that its success in doing so was a magnificent achievement. □

Sir Harry Hinsley is at St John's College, Cambridge CB2 1TP, UK. During the Second World War he worked in the UK Government Code and Cypher School.

Genetics besieged

David Joravsky

Lysenko and the Tragedy of Soviet Science. By Valery N. Soyfer. Rutgers University Press: 1994. Pp. 379. \$39.95.

As soon as Soviet intellectuals probed Gorbachev's *glasnost* and discovered that it was for real, rage erupted, blowing away all semblance of respect for the Soviet system. This history of the Lysenko affair emerged in that collapse of conformism, that eruption of fury, which had been

extremes, to ground moral judgement on deep probing of historical cause and effect — in short, to get beyond melodrama and discover history as genuine tragedy.

Candid analysis of "schizoid" elements in his own career — to take an obvious example — might have illuminated the patron-and-client pattern that helps to explain the great paradox of Soviet science after Stalin: an ebbing of ideological intrusion, a great increase in the number of scientists, and a marked decline in creativity. Soyfer, as I read the record, was a protégé of N. P. Dubinin, a geneticist who defended his embattled discipline under Stalin and led its rebuilding afterwards,

culture. Bosses and biologists shared the dream of quickly "overtaking and surpassing" the US Department of Agriculture, whose network of research centres and "extension services" proved the beneficent power of government and science working together for the advancement of farming. That dream inspired the creation of the Lenin Academy of Agricultural Sciences, the organizing centre for the legendary labours of N. I. Vavilov, who did not protest when his "law of homologous series" was acclaimed as the biological equivalent of Mendeleev's periodic table. Soyfer repeats that claim without criticism and ignores Vavilov's forecast of biologists "sculpting organic forms at will", which foreshadowed the slogans that the 'agrobiologists' would use against him.

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To be sure, biologists' modest indulgence in romantic confusion of dream and reality was only a small source of the violent nightmare that Communist leaders brought to agriculture and to biological science with the forced collectivization of 1929 to 1932. But it was a source of that berserk leap forward into total confusion between will and understanding, the will of tyrants, the understanding of tyrannized servants. Angrily disappointed with scientists' dreams, Communist bosses turned to the nostrums of semiliterate cranks. The protracted conflict that ensued favoured Lysenko and his crew in the 1930s and 1940s and turned against them in the 1950s and 1960s. "The Party" — never the monolith it pretended to be, always a complex body in evolution — moved from expectations of miraculous aid from science to pretensions of miraculous aid from 'agrobiology', and finally to the realization that no miracles are to be found, whether in science or pseudoscience, that might help violent bosses to modernize the peasant agriculture of an underdeveloped country.

The first solid history of this process was written by Zhores Medvedev, a dissident of the 1960s who really was cast out of his native land for insistently criticizing its regime (*The Rise and Fall of T. D. Lysenko*, Columbia University Press, 1969). The other standard history, worked out in greater detail on a broader canvas, was done by myself, an outside observer who mined the Soviet record and interviewed participants of both 'camps' (*The Lysenko Affair*, Harvard University Press/University of Chicago Press, 1970/1986). Soyfer pays scant attention, mostly dismissive, to Medvedev's book, and none at all to mine — except to accuse me of crediting Lysenko's earliest work with scientific merit. If he had read my book, he would have seen that I did nothing of the sort, and, more importantly, he might have found his way out of Kremlinology, the explanation of Soviet history by appeal to the little minds of

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Agricultural propaganda — a Soviet poster showing the benefits to be reaped from the "Cultivation of the Virgin Lands" (1950).

accumulating for a long time. Beginning in the 1950s the author had been making a Soviet career as a molecular biologist, while also quietly collecting materials about the 35-year war that "agrobiologists", as the Lysenkoites called themselves, waged against genuine biology. Soyfer reached his breaking point in 1980, before *glasnost*, when he signed a dissident petition on behalf of Sakharov, was fired from his job, and turned to the writing of *Power and Science*, as this book was originally called. *Glasnost* enabled him to publish a chunk of it in a Soviet mass circulation magazine (also in *Nature* 339, 45 and 340, 732; 1989); the whole Russian text appeared in the United States — and there, at George Mason University, he has wound up as an émigré.

He pictures himself as a leader of "the defiant outcasts" as early as the 1950s, while other people felt "forced to lead schizoid lives", pretending to revere a system that they actually loathed. His story is constructed of such dichotomies; strong, weak; smart, dumb; heroes, villains; praise, blame. He is too excited to explore the gradients between dichotomous

but earned the contempt of other scientists by his grotesque self-conceit. Soyfer echoes this contempt in the present book, but a history of genetics that he published in Moscow in 1970 (*Ocherki istorii molekuliarnoi genetiki*) exalted Dubinin in its grandiose conclusion that genetics would bring evolution under human control, exchange Earth's living forms with other worlds, explain consciousness, create thinking animals, eliminate defective human genes and master "directed mutagenesis, which will place the organic world in the power of man" (p. 226).

Pseudo-Promethean blather was a characteristic of 'agrobiology' in the Stalin era, as Soyfer scornfully shows. What he does not stop to ponder is the contribution of genuine biologists to exaggerated hopes of benefit from applied science. Such vacuous vapouring as he learned from Dubinin in the post-Stalin era was merely amusing, without influence on men of power, as far as I know. The serious problem was the enthusiasm of the early years, when revolutionary leaders rallied genuine scientists to the enormous task of modernizing Russia's peasant agri-

Stalin or Khrushchev and to intrigues at their court. Intermittently Soyfer declares that "the system" was to blame for Lysenkoism, but he does not get beyond the empty declaration. He does not analyse 'the system' in the process of historical development, or confront the difference between analysis of cause and effect and assignment of blame or credit. Soviet histories were written this way before *glasnost*, in reverence instead of rage. Now pluses and minuses have been reversed, but the crude algorithm persists.

Official Soviet histories credited the Communists with modernizing a backward country; Soyfer blames them for ruining "a once bountiful land, with the world's largest sown area and pasturage", for destroying "the well-organized system that Lisitsyn, Vavilov and others had established to maintain pure seed production" and wrecking "the leading position that Russia once held in genetics". It is painful to see fantasy still projected upon Russia's agricultural realities — in retrospection now instead of anticipation — and embarrassing to read Soyfer's boasts of Russian priorities in genetics, which recall claims that provoked worldwide laughter not long ago. I am not doubting that genetic science made remarkable progress in Russia during the 1920s. I am merely noting that it did so under a Communist regime, while applied science in agriculture did not make such progress. Both the official histories of the Soviet period and the simplistic inversions of *glasnost* historiography ignore the hard question: why did revolutionary commissars repeat the forceful top-down pattern of modernization that began with Peter

the Great in the early eighteenth century, intensifying servile relationships and the frightful inefficiencies that accompany them?

Soyfer's book is nevertheless a useful addition to the scholarly literature. He has done some digging in archives and summarizes a good deal of what others have uncovered. More significant are the materials he collected over many years from some of the major participants, although he is sometimes naive in repeating their stories as gospel. For example, the tale of Stalin's final interview with Vavilov, which reached Soyfer at fourth hand almost 50 years after the event, has the style and substance of Soviet folklore.

Soyfer's most striking achievement is his portrait of Lysenko, whom he observed and interviewed at length over a considerable period of time. Semiliterate, charismatic, fanatic, wilfully contemptuous of careful reasoning with hard evidence — all that has been said of Lysenko in previous works, but never with the persuasive detail and intimate evidence that Soyfer provides. He even sympathizes with the brute in his fall from power, since the "personal drama had nothing to do with the fate of Lysenkoism as a social phenomenon". This simplistic disjunction is unwittingly revealing of the extreme separation of power from responsibility that has plagued the Russian system for a very long time, even in the minds of those who rebel against it. □

David Joravsky is in the Department of History, Northwestern University, 1881 Sheridan Road, Evanston, Illinois 60208-2220, USA.

Abamp to zyometry by way of mingel

Walter Gatzert

The Dent Dictionary of Measurement. By Mike Darton and John Clark. *Dent*: 1994. Pp. 538. £30.

"ALL knowledge", Dr Johnson declared, "is of some value. There is nothing so minute or inconsiderable, that I would not rather know it than not." I wonder though whether even he might not have drawn the line this side of being instructed that the Danes measured dry volumes by the *tønde*, one of which was the equivalent of 144 pots, 8 *skaeppe* or 4 *fjerdings*; or that in ancient Russia there were 40 *funte* to a *pud* and 30 *pudi* to a *packen*. Consider furthermore that in the Iberian peninsula — I have of course only Messrs Darton and Clark's word for it — an *almude* (that is to say 16 *octavillos*) is one-twelfth part of a *fanega*, but whereas the Spanish *fanega* makes up 55.50 litres (or, as Darton and Clark helpfully add, 12.21 UK gallons or

14.66 US gallons), the Portuguese *fanega* is no more than 55.364 litres and so forth.

Darton and Clark have given us more than 500 pages, overflowing with such revelations. There is masses on sport: you can satisfy yourself as to the dimensions of tennis courts, of tennis rackets or of cabers, and you will discover, if you persist, the significance of the lag line in the ancient sport of marbles. There are some 130 collective nouns — a *smuck* of jellyfish but a *doult* of pigs (wild), and as for snipe they come in *walks* when on the ground but in *wisps* when in flight. ("Over there, Mellors, a wisp! Quick, the twelvebore (0.729 – 0.740 inches)" .) If, incidentally, you bag a brace, you are actually securing a *bras* — one to be held out in each arm. (The next entry is the Bragg angle: but we know all about that.)

Our lexicographers are also strong on music and are generous with facts about modal scales, key signatures, standards of

pitch and the like, not to mention the ranges of instruments — including the B-flat alto flugelhorn (2½ octaves from E below middle C, whereas a psaltery will comfortably manage 4 octaves).

There is of course plenty to bemuse the scientist, such as instruments for every imaginable measurement, and especially units — units current, units defunct and units that never caught on in the first place; so while the *slug* survives amongst the pounds and the feet, the *glug* was still-born as the metric unit of mass, nor did the *inferno* find favour as a measure of the temperature of stars. The dollar is not only a dollar (etym.: *thaler*, from *Joachimsthaler*, a coin minted from silver, mined in Sankt Joachimsthal, now Jachymov in the Czech Republic, first issued in 1519), but also a unit of reactivity, equal to that contributed by delayed neutrons. Pain, it seems is measured by the *dol* (in a dolorimeter) and herrings by the *cran*.

The standard of accuracy of the definitions is, so far as I am able to judge, high, though the odd mistake does crop up: the Bohr magneton, for instance, does not come in joules per kelvin, the rate constant of a reaction is misdefined, there is a wholly inscrutable entity called a *biomolecule*, and surely nobody now believes that the direction that water swirls down the plug-hole changes at the equator in accordance with the Coriolis force? Tested for comprehensiveness the dictionary came through triumphantly; I looked up first the Scoville scale, which measures the hotness of chilli peppers, and was rewarded with the information that a standard chilli registers about 5,000°, and the incandescent kind 8,000°. Among the few units I failed to find was the hardness scale for pencils, and neither was there any entry under *degrees Twaddle*, which (if memory serves) measure alkali titre. There is, on the other hand, a great deal of repetition: the seven sizes of wine bottles from the magnum to the nebuchadnezzar are all tabulated under each, there is the half-mile ("see half a mile"), as well as the mile with all its constituent parts, and units tend to recur under milli, micro, centi and the rest — but oddly, the smallest is atto, as in attomole, and zetto does not appear (though it is true that it is very small). I cannot remember which physicist it was who suggested that zetto may have originated as a misprint for zeppo, after the forgotten Marx brother, and that the zettomole should therefore be followed by the chicomole, the harpomole and the grouchomole. (As the last corresponds to less than one molecule, it could also perhaps be termed the benveniste.)

So, then, if you are not stirred by the discovery that 1 *schoff* contains 10 *charki*, while 10 *schoffs* make a *vedro*, and more, that the *schoff* (of which there are 0.9259 in two UK pints, though only 0.7722 in two US pints) is identical to the old Dutch

mingel (whether by coincidence or design no one knows) then I would not urge you to buy this book. But Flann O'Brien, who spent his literary life pushing satire to its outermost limits, would undoubtedly have drawn much inspiration from its pages. And one final consideration: it was said that when Aldous Huxley was passing through his phase of reading the *Encyclopaedia Britannica*, so ravenous was he for knowledge, his conversation would for one excruciating month be confined to topics ranging from *Brassica* to *Caernarvon* and the next to anything from *Caesar* (*Gaius Julius*) to *Cement*. The *Dent Dictionary* could be put to the same use and so might help ensure that one's dinner guests do not linger too long over the port and cigars. □

Walter Gratzer is in the MRC Muscle and Cell Motility Unit, King's College. 26-29 Drury Lane, London WC2B 5RL, UK.

Wrangling in Washington

Harvey Brooks

The President's Scientists: Reminiscences of a White House Science Advisor. By D. Allan Bromley. Yale University Press: 1994. Pp. 232. \$28.50, £20.

In 1988 Allan Bromley was appointed to the two-hatted position of Special Assistant for Science and Technology to President George Bush and Director of the Office of Science and Technology Policy (OSTP). Most members of the presidential staff were already in place. The status and influence of the presidential science apparatus was then at an all-time low, having shrunk from a staff of 50 and an annual budget of \$4 million at the end of the Carter years to a staff of 11 and a budget of \$1.5 million under Reagan. Even when the President's Science Advisory Committee and the position of science adviser were abolished by Nixon in 1973, and the responsibilities of the Office of Science and Technology (OST) were returned to the director of the National Science Foundation (NSF), staff support available within the NSF for roles previously assigned to the OST exceeded that available to the director of OSTP in 1988. Moreover, relations between OSTP and Congress were grimmer than ever. Yet by end of the Bush administration in 1992, the OSTP staff had grown to 65 and its budget to \$6.2 million, and it had become the largest agency within the Executive Office, with excellent congressional relations and good collaboration with the Office of Management and Budget. The result? Many presidential initiatives and a

programme and capability that turned out to be the foundation for most of the policies and programmes selected for rapid expansion by the incoming Clinton administration.

This book is a highly personal and candid account of how all this came about. It provides a fascinating new chapter in the rocky history of science and technology in the White House, a history that began in 1951 under President Truman. Bromley gives valuable insights into the institutions and inner workings, formal and informal, of government at the level of the White House; it should be compulsory reading for anybody wanting to really understand how science policy is made: a good deal of the material here cannot be found in the official documentary sources usually used by policy scholars. This is particularly true of the chapters on budgetary process and the interactions between Congress and the Executive. The book is full of surprisingly frank anecdotes and comments on people active in debates about science policy and especially technology policy in the upper reaches of the executive branch of the Bush administration. The picture of national policymaking that emerges is not always very flattering, replete as it is with turf battles and oversensitive egos, although negative and positive comments are fairly evenly balanced. The heroes and villains are not always the same as those identified by the contemporary press.

The inherent problem of science and technology in the policy process arises from the fact that they are crucial inputs to policy debates that are primarily non-technical in character. At the same time, when budgetary issues are involved, it is difficult for the OSTP to avoid the appearance of representing the special interests of the scientific community that is uniquely dependent on federal financial support in competition with other political and economic constituencies, especially since the discretionary part of the federal budget has shrunk from 70 per cent of the total in 1960 to 37 per cent in 1993. Also, the credibility of the OSTP in all other aspects of the policy process depends on its reputation for impartiality and freedom from the suspicion of being beholden to any external constituency — a difficult balancing act that Bromley seems to have carried out with unusual skill.

Bromley is at his best when dealing with issues in which he was directly involved. For example, there is a brief discussion of the controversy over the Super-sonic Transport Program in the early 1970s and of early controversies about automobile emissions. While I happen to agree with Bromley's policy conclusions on these subjects, his discussion of the underlying science is oversimplified and partly wrong. Similarly, his explanation of the reasons for Truman's veto of the original NSF legislation is a serious oversimpli-

fication of a much more subtle and complex controversy.

Indeed, the book often disappoints when it deals with the scientific basis of policy issues. For example, there was much (in my opinion unfair) criticism of the Bush administration before the United Nations Conference on Environment and Development in Rio de Janeiro in 1992 because of the administration's refusal to agree to specific targets and deadlines for the reduction of greenhouse-gas emissions. I believe that this position was absolutely scientifically defensible, but the justification that Bromley gives is vague and unconvincing.

Comparisons of US science policy with those of Europe and Japan also seem unduly simplistic and in some cases wrong. Support for science and technology in these countries, Bromley says, is centred on a single agency, in contrast to US pluralism. In fact, defence, space and atomic energy tend to be administered separately in virtually all major countries. He also states that "essentially all" research and development (R&D) goes into this creation of "new knowledge" except in the United States, where 54 per cent funds the development of large public technological systems, mainly relating to defence, space and nuclear energy. Although there is a grain of truth here, these observations are so oversimplified as to be quite misleading.

Moreover, statistics on international comparisons are presented almost entirely in terms of ratios of total R&D investment to gross domestic product, which virtually ignores the fact that US R&D investment in absolute terms exceeds that of any of the five other major countries. No credit is given to the potential advantages of economies of scale in R&D in the United States and the greater diversity and competition of ideas made possible by the sheer size of the country. No mention is made of the fact that in Germany and Japan a much higher fraction of total national R&D (65 per cent and 83 per cent respectively) is financed by private companies with their own funds than is the case in the United States (50 per cent). This may possibly explain the outstanding economic performance of the first two countries. □

Harvey Brooks is in the John F. Kennedy School of Government, 79 John F. Kennedy Street, Harvard University, Cambridge, Massachusetts 02138, USA.

■ *The Fifth Branch: Science Advisors as Policymakers* by Sheila Jasanoff has just been published in paperback by Harvard University Press (\$20.25, £13.50). "A provocative and original work...Jasanoff has pioneered the exploring of the workings of the gears and sprockets of the Fifth Branch [of government]," wrote Daniel S. Greenberg in *Nature* **349**, 116 (1991).

Feeling the difference

Stuart Sutherland

Descartes' Error: Emotion, Reason and the Human Brain. By Antonio R. Damasio. Grosset/Putnam: 1994. Pp. 312. \$24.95.

EMOTION is almost impossible to define, there is no agreement about what it is, we do not know what it is for and we are unsure what to include in it — are hunger, boredom and curiosity emotions? It is small wonder that cognitive science, neuroscience and artificial intelligence have largely ignored it. *Descartes' Error* is a spirited attempt to rectify this omission.

Antonio Damasio believes unexceptionably that emotions involve cognition, efferent output and feedback from the resulting changes in the autonomic and hormonal systems, and in the skeletal musculature (as in a smile or a frown). These effects are accompanied by the release of neurotransmitters in the brain. Damasio distinguishes primary emotions (which are innately triggered and include hunger, fear of snakes, and sexual desire) and "secondary ones", which he believes result from the formation of "systematic connections between categories of objects and situations... and primary emotions".

So far, so good: the account is well argued, but not particularly novel. Damasio's original contribution is his hypothesis about what emotion is for. The evolutionary value of the primary emotions is obvious. He argues, however, that the bodily feedback — which he calls a "somatic marker" — from secondary emotions prevents anyone making a decision from contemplating possible courses of action that would lead to bad consequences. At first this sounds far-fetched, particularly as much decision-making does not involve strong feelings. But Damasio meets this objection by postulating that the brain can bypass feedback from the periphery by itself bringing about patterns of activity similar to those that genuine sensory feedback produces: he terms this an "as if" emotion.

He gives impressive evidence for his suggestion. Characterizing the defects that arise from prefrontal lobe lesions has for long been a difficult problem. One of Damasio's own patients who was subjected to a large number of mental tests, including tests of intelligence, personality and moral reasoning, was normal on all of them. Yet his behaviour was far from normal. He was so distractible that he was unable to hold down a job and he vacillated wildly over personal decisions. Furthermore, his emotions were shallow. Damasio concludes that because in this patient and other people with prefrontal lesions the consideration of bad options is not nipped in the bud by an emotional reaction, they are incapable of taking and

sticking to decisions. Moreover, as he observes, the prefrontal cortex is ideally suited to combine emotional feelings and cognition, for it receives an input from the sensory areas of the brain, including the somatic areas, from association areas and from those parts of the limbic system known to be implicated in emotion and primitive drives.

Damasio's most interesting evidence, however, comes from two experiments in which he compared the galvanic skin response of normals and prefrontals. In his first experiment, he showed his subjects pictures, most of which were emotionally neutral, but some of which were scenes that would be expected to arouse strong emotion. To the latter set of pictures, normals gave large galvanic skin responses, whereas the prefrontals gave none, even though they could describe the emotions that the pictures would normally produce, such as fear, disgust or sadness. These results suggest that the prefrontal lobe is implicated in secondary emotion, for the prefrontals gave normal galvanic skin responses to stimuli producing primary emotions, such as a startle stimulus.

The second experiment lends even more direct support to Damasio's hypothesis. Subjects were asked to select a card from one of four piles. Initially they received a reward if they picked a card from the first two of the four piles and a lower reward if they selected cards from the other two. Subsequently, they sometimes suffered a very large loss if they picked cards from the first two piles. Normal subjects correctly switched to the less risky piles, prefrontals did not. Damasio concludes that it is the somatic marker that prevents the normal subjects from picking cards from the dangerous piles: prefrontals continued to do so because the emotional warning of danger was absent. This conclusion should be treated with caution since it is known that prefrontal patients are bad at switching responses even in situations where little or no emotion is involved.

There were, however, two further important findings. After their first experiences of large losses but before they were conscious that some piles were more remunerative than others, the normal subjects would sometimes pick a risky card and on these occasions they showed a galvanic skin response immediately before doing so. Damasio suggests that the emotional response (the somatic marker) had been learned unconsciously before the subjects were consciously directing their

choice correctly. The operation of somatic markers on unconscious thought processes would explain correct intuitions: mathematicians tend to consider consciously only lines of thought that might be fruitful; the others are unconsciously blocked by somatic markers. Finally, it is of interest that although the prefrontals persisted with a poor strategy, some of them were later able to say that the risky piles were bad and the others better: conscious knowledge is not enough to direct choice in the absence of somatic markers.

These fascinating results (and some equally striking findings obtained since his book was written) provide strong support for Damasio's hypothesis. Moreover, in addition to the evidence adduced by Damasio himself, there are some surprising findings — from a different field — that suggest emotion is involved in cognition: people are better at remembering and recognizing faces when they are instructed to concentrate on their emotional impact rather than on their physical form.

Damasio's own arguments are ingenious and wide ranging: they have been given only the barest sketch here. Only neurophysiological work could disprove his theory, because of his introduction of that Catch 22 — unobservable "as if" emotions. A cynical psychologist might argue that Damasio's basic hypothesis is that people perform actions that are likely to have agreeable consequences and avoid those that have disagreeable ones, but Damasio could rightly retort that it is the neurophysiological mechanism that is important. His thoughtful and modest exposition should be taken seriously. Apart from illuminating the function of parts of the frontal lobes, he has proposed a new physiological mechanism that is likely to be much investigated over the next few years. It is no mean feat to say something original and intelligible about emotion. □

Stuart Sutherland is in the Laboratory of Experimental Psychology, University of Sussex, Brighton BN1 9QG, UK.

New in paperback

The Life of Isaac Newton by Richard Westfall. Canto (Cambridge University Press), £7.95, \$11.95. An abridged version of his monumental biography *Never at Rest*, first published in 1980. "Westfall is a lively and lucid expositor of Newton's ideas and he has written an excellent book for those who want an authoritative introduction" (William R. Shea, *Nature* **364**, 681; 1993).

The Golem: What Everyone should know about Science. By Harry Collins and Trevor Pinch. Canto (Cambridge University Press), £5.95, \$9.95. "Perverse but entertaining... the writing is deft, the stories are good, and there is not a boring page" (Walter Gratzer, *Nature* **364**, 22; 1993).

Shadows of doubt

P. W. Anderson

Shadows of the Mind: A Search for the Missing Science of Consciousness. By Roger Penrose. Oxford University Press: 1994. Pp. 457. £16.99, \$25.

ABOUT 15 years ago, Roger Penrose's former student, Stephen Hawking, devoted part of his inaugural lecture as Lucasian professor in the University of Cambridge to the prediction that by the year 2000 physicists would have been made obsolete by electronic computers. Although Penrose does not refer to this particular instance, it would seem that it is his concern about this kind of optimistic view of the capabilities of computers that motivated him to write *The Emperor's New Mind* and now, some five years later, its sequel.

In *Shadows of the Mind* he elaborates on his earlier proposals and attempts to answer his critics. I was put off reading *The Emperor's New Mind* by the many critical reviews it received, so I came to the sequel fresh, albeit prejudiced. Let me say without hesitation that my prejudices have been amply confirmed. Nonetheless, reading this new book is a fascinating and mind-stretching exercise. I can imagine that the average scientific reader, unfamiliar with the many-faceted mental universe that Penrose inhabits, will be dazzled by his extraordinary breadth and scope. But the more extraordinary the mind, the more unfortunate it is when it is used to entertain what may well be vain speculations. Also, Penrose's great reputation, charm and skill as a writer (perhaps not as evident in this book as in the previous one) should not blind us to the fact that his professional background is not really relevant to his subject matter.

The book consists of two parts. In the first part he argues that the mind does things that are beyond the capabilities of a "mere" computing machine. (The word "mere" is a trap; in this case it is in the meaning of "mere" that the meat of this statement lies.) This is why machines are not about to replace physicists (or mathematicians). I heartily agree. But he then concludes that some novel laws of physics must be crucial to the operation of our brain, and that they might possibly relate to certain aspects of quantum gravity theory. This conclusion troubles me. In the second part of the book, Penrose goes on to discuss his view of the gaps in our understanding of physics and biology through which such radically new material could creep into the theory of the brain. I can find little here to sympathize with.

He presents four alternative propositions about the mind that are intended to cover all possibilities: *A* is the "mere" machine; *B* is the machine with an im-

posed "Des Cartean" observer, which as far as its method of operation is concerned is essentially *A*; *C* uses possible new laws of physics; and *D* is the supernatural alternative that does not obey natural law. Penrose rejects *D* as a cop-out, as an alternative inappropriate to a scientist. *A* and presumably *B* are excluded by very subtle and ingenious reasoning involving a restatement of Gödel's theorem as an argument about computer algorithms: that no "provably sound" algorithm operating on a Turing machine or equivalent computer could ever encompass all the correct mathematics of which the human brain is capable.

To my mind, the most likely alternative is different from all of these; it is that the operation of the mind follows the ordinary laws of physics and chemistry, without

discontinuous events with continuous ones. And most of all, the mind experiences objects: complexes of data that exist in space-time and show different aspects of the same entity.

Second, the brain's connections and its program are not complete until after it knows that other autonomous entities — other similar machines — constantly surround and communicate with it. There is a hint: the primitive mind animates with purpose even those objects in its surroundings that are inanimate. Communication is impossible without two factors: someone to communicate with and a common perception to communicate. Communication is a primary feature of mind, which is currently thought to be already established before the mind is complete.

Third, there is a fair amount of evidence that the mind is not a single, simple entity: it may be a number of independent, autonomous systems squabbling for a central dais. Multiple personality disorder is only an extreme form of what goes on in the mind all the time. There is no single Turing machine or single tape. It is not clear that it really is correct to model a parallel collection of semi-independent machines that is, in some sense, wider than it is deep, in terms of a sequentially operating single algorithm. In discussing complexity, this can be a different 'large-N limit', with different capabilities.

Fourth, some of Penrose's arguments, and much of computer theory, are about exact, rigorous solutions. His computers do not 'halt' until they have found an exact answer. This can be crippling. In the real world it is usually adequate to 'satisfice', to use Herb Simon's term. Methods directed merely at finding an acceptable way to do something can be much more efficient than exact ones. This is one way the mind can take advantage of its knowledge of the structure of the world.

As I see it, it is not really necessary to identify what particular aspects of the nervous system allow it to evade the rigid, rigorous, logical arguments with which Penrose tries to pin it down. One has merely to point to the remarkable ability of complex systems to develop emergent properties that overcome the apparent limitations of their separate constituents. Apparently rigorous 'theorems' that seemed to make antiferromagnetism as well as superconductivity impossible turned out to be irrelevant in the face of the emergent property of broken symmetry, just as all the many kinds of argument against evolution — the thermodynamic one, for example — do not prevent its happening. What does seem clear is that the above, and other new concepts and methods using conventional physics and chemistry, are far more likely to solve the problem of mind than is quantum gravity.

It is impossible to analyse here in detail all of the arguments in the second part of

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Consciousness explained? Cross-section of a flagellum, showing its internal arrangement of microtubules.

bizarre additions, but that it operates using algorithms, concepts and mechanisms that are quite outside the system of apparently rigorous 'theorems' of computer theory. In computer complexity theory, for instance, the complexity classes are often meaningless categories. But by using one's knowledge of the nature of the problem to be solved, one can often do what from the theory seems to be impossible, whereas nominally 'equivalent' problems turn out to be inaccessibly distant from each other.

There are many ways in which computational methods using quite ordinary physics might evade the apparently 'rigorous' limitations of the von Neumann/Turing architectures. What follows are just a few, mainly culled from various recent books and articles about the mind.

First, the mind's hardware is by no means complete at birth. Not only its instruction kit, but its internal and external connections are constructed using knowledge of the nature of the actual world it will function in. The concepts of space-time and of objects moving continuously in space are built in; in fact, the most obvious optical illusions involve replacing

the book. Let me pick out a few about which I have some independent knowledge. The long section on quantum measurement theory emphasizes the many dilemmas and queries that one encounters if one assumes, with Bohr, that there is a genuine dichotomy between the microscopic world in which quantum theory applies and the macroscopic world of measurement apparatus. These mind-bending difficulties ('EPR', 'entanglement', Bell's theorem and so on) are the stuff of rather boring philosophical discussions; it is hard to see how they could make consciousness easier to understand. But one seems unable to find any natural scale for this dichotomy, among other things; and many, if not most, thinking quantum physicists reject the idea that there is any dichotomy, and assume that quantum laws hold all the way up and down. This possibility is dismissed by Penrose in two brief pages (pp. 310–312).

Penrose's primary objection to this point of view is that it is "unsatisfactory" in that it involves continual splitting of the wave function of the Universe into fragments, only one of which an observer can perceive. (This splitting is the 'many-worlds' viewpoint, although there are other ways to interpret the same mathematics, among them that of M. Gell-Mann and J. Hartle.) We cannot decide for nature which of her ways are 'satisfactory' or 'unsatisfactory'; that is nature's call.

More seriously, Penrose makes the claim that there is no quantitative justification for the all-quantum viewpoint. In a popular book, *The Quark and the Jaguar*, published earlier this year, as well as in several articles, Gell-Mann discusses at length the rapid and complete 'decoherence' between alternatives, which prevents the observation of coexistence within, for a typical case, 10^{-21} seconds, by actual and precise calculation. That this is a consistent and logically satisfactory possibility has been obvious for many years, since Fritz London first proposed it in 1938. It has now been formalized. Penrose should have been aware of this.

With regard to superconductivity, Penrose has, I think, got the implications of macroscopic quantum coherence backwards. In a superconductor, the quantum field itself becomes a macroscopic object, a perfectly measurable, rigid, thermodynamic parameter of the body on the same footing as strain, torque, entropy or magnetization, and obeying the same general laws (which derive from the general phenomenon of broken symmetry). Coherence is maintained not by an energy gap as Penrose suggests, or by some mysterious persistence of a quantum superposition, but by mundane thermal equilibrium. It has always seemed to me that for anyone in possession of the facts about superfluidity and superconductivity, it would be hard to doubt that classical

behaviour is simply large-scale quantum behaviour — that is, an emergent property of large quantum systems. But habits of thought die hard.

Microtubules are for Penrose the likely seat of the mysterious quantum gravitational effect that makes the mind possible. Biophysicists who specialize in their study would agree that the behaviour of microtubules is indeed interesting and complex, but would see no need (nor in fact any room) for anything but the characteristic chemical control mechanisms with which we are familiar.

Penrose has written a complex, erudite and fascinating book, and my complaints about it do not mean that I did not enjoy and learn a great deal from reading it. But one should keep in mind that Penrose is a mathematician with little experience of the messy, frustrating but ultimately deeply satisfying process of checking his ideas against the experimental facts about nature. Mathematicians are used to game-playing according to a set of rules they lay down in advance, despite the fact that nature always writes her own. One acquires a great deal of humility by experiencing the real wiliness of nature. □

P. W. Anderson is in Department of Physics, Jadwin Hall, Princeton University, Princeton, New Jersey 08544, USA.

Mental pictures on the brain

Zenon Pylyshyn

Image and Brain: The Resolution of the Imagery Debate. By Stephen M. Kosslyn. MIT Press: 1994. Pp. 516. \$45, £40.50.

THIS book is intended first and foremost as the final solution to the so-called 'imagery debate'. Its focus on this polemical task, however, seriously detracts from its potential usefulness as a study of the relation between visualization and vision, particularly from the perspective of clinical neurology.

Many of us believed that the debate, at least in the form revived in this book, had quietly disappeared as it became clear that there were serious problems with notions such as that mental images 'depict' or 'resemble' something or 'have spatial properties'. But Stephen Kosslyn now feels that these notions can be rehabilitated because "by turning to the brain, this debate can be resolved to the satisfaction of most people". But the basic problem still stands: as long as the research questions continue to be ill-posed, the problem about mental images will remain unsolved, regardless of how much brain (or other) data is collected.

Discussions of the nature of mental imagery have invariably equivocated between two very different views of what an image is. The literal option is that an image is some sort of mapping (usually viewed as a quasi-photographic projection) of the imagined scene onto some real (presumably neural) surface, possessing such physical and/or geometrical properties as shape, length, area and size. I do not know anyone who explicitly endorses this literal 'picture' option. In a way this is too bad because it is the only option that actually addresses much of Kosslyn's data (such as the increased time it takes to scan greater imagined distances or to examine smaller images). It is also the only option that clearly connects with most of the neurological findings discussed in the book. (Imagining something large, for example, results in brain activity over a larger area of cortex than imagining something small.)

The second option is that we have some 'functional equivalent' of pictures in our brain. Kosslyn talks about a "functional space" where images do not actually 'have' properties such as size or orientation but merely 'specify' them. But this option has no explanatory power because it fails to constrain the nonliteral 'image' to have any particular intrinsic properties — gone are depiction and resemblance, as are any constraints on how geometrical properties are represented. To account for empirical data one must of course reintroduce whatever additional constraints one needs, but these are no longer intrinsic properties of the image. As Kosslyn remarks, the critical properties are not inherent in the image but in how it is 'read'. Moreover, since such a functional image contains "previously digested information", there is no reason why 'reading' it should involve the visual system.

A good example of this sort of extrinsic stipulation of constraints is Kosslyn's use of a matrix as a functional image in his computer model. Notice that a matrix, by virtue of being a data structure, does not require scanning to proceed through adjacent cells, nor does it inherently preserve geometrical properties over transformations such as translation and rotation. Such constraints must be additionally stipulated. Consequently, appealing to the matrix itself does not explain predictions derived from such stipulated constraints, as it would if we had taken the literal option and assumed a surface constrained by the laws of physics.

The basic problem is that any theory of mental imagery has two fundamental degrees of freedom between which it can trade off in addressing the data, since the theory specifies both the nature of the image and the nature of the process that examines it. If we assume a literal view of the image, the physical geometry of the display allows us to make sense of some

behavioural and brain data, but only if we also assume a literal 'mind's eye' that is very much like a real eye, complete with a pattern of spatial resolution, a visual angle and a way of scanning with mental 'eye-movements'. On the other hand, if we take the nonliteral option, then empirical results otherwise attributed to the geometry of the image and the 'mind's eye' must now be built into the interaction between the image and the access process. Because there are no independent constraints on such a process, we are free to tailor it to fit the data. But we do pay a price because now there is nothing left of the idea of a depictive, geometry-preserving pictorial entity, and so there is no need to involve the visual system.

Kosslyn's book is at its best when it skirts the question of the nature of images and focuses instead on the commonality of vision and visual imagery. Both behavioural and neural evidence is presented that suggests that vision and imagery may use common brain mechanisms. Although the evidence is far from unambiguous (especially evidence from the superposition of images and percepts, for which there is a simple attention-based 'indexing' explanation) it is nonetheless intriguing and worth examining in detail. On the other hand, this approach belies the goal of the book: it is irrelevant to the debate about the nature of images.

Nobody doubts that some brain mechanisms are involved in both vision and imaging. The interesting question is which mechanisms they share, because the answer might illuminate what, if anything, is special about visualizing as opposed to general reasoning. If it could be shown that imagery uses mechanisms that are specific to vision, then we would have discovered something of considerable interest — even though it would still not address the traditional 'imagery debate'. Mechanisms specific to vision might, for example, include those responsible for the segregation of figure from ground, spontaneous construction of three-dimensional percepts from two-dimensional contours or motion cues, or any of the many distinctly visual phenomena described in textbooks on visual perception. Kosslyn's book provides no unproblematic evidence implicating this kind of mechanism. What it provides instead is some speculation about why positing internal pictures helps to explain perceptual invariances and the integration of information from glances — speculation that helps only to perpetuate the misleading subjective experience we have that when we see or imagine something we are creating a replica of it in our heads, an idea that has surely run its course over the past two millennia. □

Zenon Pylyshyn is in the Center for Cognitive Science, Rutgers University, New Brunswick, New Jersey 08903, USA.

Minds possessed

Susan Blackmore

Dark White: Aliens, Abductions and the UFO Obsessions. By Jim Schnabel. Hamish Hamilton: 1994. Pp. 304. £16.99.

How can apparently sane, intelligent and likeable people believe that four-foot-high aliens are visiting our planet and abducting people? If you are perplexed by this question, Jim Schnabel's latest book *Dark White* (Grey — geddit?) will give you some answers.

A recent Roper opinion poll claimed that nearly four million Americans have been abducted. The stories are remarkably consistent as well as outrageous. People are woken in the dead of night or, less commonly, taken from their car or workplace, and confronted by large-headed, small-bodied, huge-eyed grey aliens who transport them magically into a spacecraft. Here they are taken down curved corridors, laid on a flat table and subjected to humiliating or terrifying mental, medical and gynaecological procedures. Eventually they find themselves back in bed but with two or three hours 'missing'.

The stories cry out for comparison with fairy abductions, incubi and succubi and myths such as the Old Hag of Newfoundland, who visits victims at night and tries to suffocate them. Schnabel deals well with all of these but his greatest strength lies in the way he portrays the main characters involved.

Take Budd Hopkins, a New York artist who first saw a UFO in 1964. He began to investigate experiences of 'missing time' and found himself overwhelmed by people needing help. Perhaps it was because he was already well known as an artist that people took him seriously. He learned how to hypnotize them and soon they were 'remembering' the abductions that took place in the missing time intervals.

Schnabel portrays Hopkins as a kindly and sincere man who really wanted to understand what was going on. The vividness and consistency of the stories persuaded him of the nuts-and-bolts reality of the aliens and their UFOs. It was also Hopkins who first came across stories of the alien hybridization programme. As Schnabel points out, as soon as Budd recognized it, women began turning up with strange scars and tales of disappearing pregnancies, and men told of having sperm removed by beautiful female aliens.

Contrasting with the sincerity of a possibly naive artist is the craziness of Whitley Strieber. The way Schnabel describes him you would not trust his opinion of what he had for breakfast, let alone the reality of his aliens. His best-selling book *Communion* followed several horror nov-

els that, according to Schnabel, mix fiction and biography with alarming ease — and apparently it was going to be called *Body Terror* until Strieber decided that it must not frighten people.

This tension between terror and enlightenment runs through the whole story. Whereas Hopkins' abductees seemed only to experience pain and fear, those studied by Leo Sprinkle, a psychologist from Wyoming, more often reported spiritual experiences and "environmental and spiritual consciousness-raising". Joining this clan was soon to be John Mack, well known as a Pulitzer prizewinning author and professor of psychiatry at Harvard University. With such qualifications he had an authority perhaps greater than any of the others involved. He soon collected a large following of abductees convinced that the aliens had peaceful intent and wanted to warn us of impending environmental disaster.

Like Mack, Ken Ring, a psychologist, sees the positive side. He had studied near-death experiences and noted the similarities between these and abductions. Both pointed to the progress of human consciousness towards unity and harmony.

A problem for anyone who simply wants to know whether or not the aliens exist is that these academics, unlike the more down-to-earth Hopkins, can evade such a crude question: after all, it depends on what one means by reality. But what of the science?

There is certainly a scientific story to be told. Yet, with no index or proper references, this book fails to tell it well enough. This is a shame because the groundwork is all there. Schnabel tackles sleep paralysis, in which the muscle paralysis of dreaming sleep can carry over into waking, and compares it with abduction myths. He considers childhood trauma, the problems of hypnosis and the arguments over false memory.

Best of all he clearly explains the most tricky — and interesting — of the theories. Michael Persinger, a Canadian neuroscientist, argues that the experiences are caused by firing in the temporal lobes of the brain and can be set off by changes in magnetic fields. Schnabel marshals the evidence for this theory and concludes that abduction accounts may all be similar not because the aliens are all similar but because our brains are. Stimulation of the relevant areas, combined with cultural and personal material, may account for it all.

The book convinced me that abduction accounts are well worth serious research. It is not a question of whether or not the aliens exist but of what the experiences have to tell us about our minds and brains. □

Susan Blackmore is in the Department of Psychology, University of the West of England, Bristol BS16 2JP, UK.

A life on the wild side

J. L. Cloudsley-Thompson

Naturalist. By Edward O. Wilson. *Island/Shearwater Books*: 1994. \$24.95.

It is difficult to read an autobiography without inwardly questioning the motives of its author. Is the writer driven primarily by a desire to share with others who are perhaps less talented, less energetic or less extrovert the experiences, thrills and rewards of a successful career, or is the exercise merely what in the 1960s was known as an ego-trip? No doubt many factors are involved, and it is probably impossible to write of oneself without being to some extent egocentric.

Somewhat less restrained than most, Edward Wilson's autobiography contains plenty of elements from both these two extremes. As one of the leading field biologists of the present century, he is, however, entitled to a certain degree of self-esteem. Moreover, he does not indulge in name dropping or, rather, the names he drops are mainly those of other well known zoologists — not of the aristocracy, nor of the politicians, film actors and other celebrities who feature so largely in many personal narratives. At the same time, his fascination with living organisms is unquestionably sincere: his book should provide inspiration to youthful biologists whose bent is towards natural history rather than the more fashionable microbiology or mathematical ecology. He is also generous with his praise of others and by no means uncritical of himself. He states frankly that he is a poor mathematician, but has "an unusual ability to make comparisons of disparate objects, thus to produce syntheses of previously unconnected information". He certainly justifies his claims to write "smoothly" and, throughout his career, to have "pushed" his considerable strengths and "skirted" his weaknesses.

Wilson does not lack courage: witness his account of a reckless youthful attempt to capture an outsize cottonmouth moccasin, nearly as long as himself, in a swamp on the Alabama border with Florida, north of Pensacola. He had previously been bitten by a pygmy rattlesnake, so he knew what to expect — and he had also earned the "Scout merit badge for Reptile Life". He became an entomologist rather than a herpetologist because, while fishing for minnows at an early age, one of the dorsal spines of a pinfish accidentally pierced the pupil of his right eye when he "yanked" his line too hard. The lens subsequently clouded over and had to be removed. He was held down while the "anesthesiologist, a woman named Pearl Murphy, placed a gauze nose cone over my nose and mouth and dripped ether into it". For years afterwards he became

nauseous at the smell of ether.

Every facet of an adventurous youth in the 'Old South' is recalled with striking clarity: the personal details of a broken home, his rapid advancement to "Eagle Scout with palm clusters, the highest rank", his experiences as a cadet in the US Reserve Officers' Training Corps, a technique for catching flies by hand, his unrealistic athletic ambitions and his determination to excel, despite any natural handicaps. He compares himself with a friend who won the over-40 laurels in the 1980 Boston Marathon in the following words: "He was Mozart to my envious Salieri". Above all, the reader will be impressed both by his competitiveness and by his insatiable curiosity. It is well said that the most important ingredient of scientific research is to ask the right questions: Wilson constructs elaborate syntheses and comes up with fresh unifying concepts.

For British readers, one of the most intriguing aspects of the early chapters of this unusual book may lie in the differences in attitudes and conventions between boys in the United States and in the United Kingdom. Whereas in the United Kingdom it was not considered 'good form' half a century ago to indulge in fist fights — even in preparatory school — in the more brash Gulf Coast Military Academy it was deemed unmanly to refuse a fight. As the narrative unfolds, with its detailed self-analysis, the reader is given a fascinating insight into the mental development and maturation of a distinguished zoologist as well as of the evolu-

tion of the field of study that he helped to define.

Biologists will probably find the second section of the book, "Storyteller", even more interesting than the first, "Daybreak in Alabama". In it, Wilson takes his readers on travels throughout the South Pacific, Australia and New Guinea. His unsuccessful search for the most primitive known ant, *Nothomyrmecia macrops*, makes a good story, as does the account of his chance discovery, in Sri Lanka, of *Aneuretus simoni*, which apparently links the Myrmicinae and the Dolichoderinae. In a chapter aptly named "The Molecular Wars", Wilson describes his clashes with James Watson who, "having risen to historic fame at an early age, became the Caligula of biology", and we are given the low-down on how Harvard University makes offers of tenure to its faculty. The pace quickens as conceptual discoveries such as character displacement and sociobiology are developed, while the final chapter is devoted to concern about the global loss of biodiversity. I found the controversy over sociobiology, especially the part played by Richard Lewontin, quite extraordinary.

This account of Wilson's life provides detailed insights into the creation of a renowned scientist, and is an elegant account of the development of his exciting ideas. It is illustrated by numerous photographs of the author, mostly while receiving well-earned medals, awards and prizes, and lively drawings by Laura S. Southworth. □

J. L. Cloudsley-Thompson is at 10 Battishill Street, London N1 1TE, UK. He is emeritus professor of zoology at the University of London and former professor of zoology at the University of Khartoum and keeper of the Sudan Natural History Museum.



SKELETON of the hindleg of a white-throated capuchin monkey (*Cebus capuchinus*). The leg joints allow the foot to be placed in different positions and at different angles. Picture taken from *Bones: The Unity of Form and Function* with text by R. McNeill Alexander and photography by Brian Kosoff. A beautiful blend of art and science. Weidenfeld and Nicholson/Macmillan, £19.99, \$40.

Look to the ant, thou sluggard

Deborah M. Gordon

Journey to the Ants: A Story of Scientific Exploration. By Bert Hölldobler and Edward O. Wilson. *Belknap/Harvard University Press*: 1994. Pp. 228. \$24.95, £19.95.

HÖLLDOBLER and Wilson have done for ants what Levis did for denim. Ants were always there, if only to be stepped on. But now it is widely recognized that ants merit the attention of any amateur naturalist, and that the growing numbers of researchers studying social insects are addressing some of the most important current problems in evolutionary and behavioural biology. In *Journey to the Ants*, the authors demonstrate how they helped to bring about this change in ant public relations. The book is a greatly abridged version of their earlier *The Ants*, but pitched at an educated lay audience.

A key to the authors' success in improving ants' public image is surely the use of intriguing illustrations that enlarge ants to a human scale. The book contains some of Hölldobler's finest photographs of these creatures, as well as some beautiful scanning electron micrographs and a variety of drawings and paintings.

Another attraction is that the authors describe ants in an appealing way. The ants in this book always know exactly what they are doing. They do not mess around; their duties and their destinies are clear. "Relentless" and "fanatical", they are "self-sacrificing...minions programmed to

act in concert", "gangs of factory workers" whose "loyalty is nearly total" and whose "lives are consecrated" to the queen's production of more ants. Thus regimented, ants accomplish advanced feats of chemical signalling, nest construction and strategic warfare. This dictatorship, however, has no dictator — not even the queen, a "demanding beggar" who, "psychologically reduced" (and physically incapable), cannot direct their behaviour. Instead, they are led into servitude by the inexorable hand of natural selection.

People have been watching ants for a long time, and the view of these insects as conscientious labourers is as old as the Old Testament ("Look to the ant, thou sluggard; consider her ways, and be wise", Proverbs 6:6). But this view is one of many. Not everyone is impressed by the efficiency of ants. Mark Twain watched a foraging ant and thought its procedure "as bright a thing to do as it would be for me to carry a sack of flour from Paris to Heidelberg by way of Strasbourg steeple". Some biologists are examining the function of ant ineptitude. They see ants as elements of a distributed computational system; indeed, recent models of this sort suggest that if ant behaviour did not have a substantial random component the colony would be unable to respond to new contingencies. Another, related approach seeks to explain the evolution not of pre-



An 'alarmed' weaver ant (*Oecophylla longinoda*) with mandibles gaping and gasters cocked.

programmed regularity but of flexibility: ants respond differently to a particular chemical in different social contexts; individuals switch from one task to another as the environment requires; access to foraging territory is constantly shifting as neighbouring colonies renegotiate. Perhaps all of these forms of behavioural plasticity help colonies to respond to a changeable world. Hölldobler and Wilson's notion of "clockwork societies" provided the starting point for a variety of perspectives on colony dynamics.

In their preface, the authors point out that they unavoidably emphasize the topics and species on which they have worked. Equally unavoidably, they describe ants in a way consistent with the research programme that they created, a programme devoted to a vision of well-regulated ant societies based on hereditary caste, and to the premise that "behavior can be broken apart into atomic units", with a chemical stimulus corresponding to each unit.

Wilson wrote recently that when asked what to do about ants in the kitchen, he always says: "Watch where you step". This book will make you want to watch not only your steps but the ants themselves. The book does not encourage you to see ants differently from the way its authors do, but it will inspire you to look. Wilson and Hölldobler have each made fundamental contributions to the study of the physiology and evolution of social behaviour, and *Journey to the Ants* is an accessible and interesting guide to their approach. □

Deborah M. Gordon is in the Department of Biological Sciences, Stanford University, Stanford, California 95305-5020, USA.



Honeypot ants (*Mysmecoystus mimicus*). Both pictures on this page are reproduced from *Journey to the Ants*.

Tragic dilemmas

James M. Jasper

The Monkey Wars. By Deborah Blum. Oxford University Press: 1994. Pp. 294. \$25.

No matter how familiar they have become, the cognitive capacities of non-human primates still impress us. Chimpanzees can learn more than a hundred words in sign language or keyboard symbols, and they can piece them together in new ways. Lacking a symbol for cucumber, one asks for a "green banana". Another, introduced to fellow chimps for the first time, is unimpressed, dismissing them as "bugs... black bugs". Those who have mastered sign language use it to talk to themselves when alone, to other chimps, even to their inanimate toys. They can make the remarkable leap of classifying words. Two of them, when asked to group 17 nouns as either tools or food, made only one mistake between them. One chimp said that sponges were food. But, it turns out, this chimp regularly gobbled up sponges that were used around his compound to soak up spilled soft drinks.

Because chimpanzees and humans share 98.5 per cent of their genetic make-up, the former's exploits may not be surprising. The other 1.5 per cent can look very large or very small. But lesser primates such as rhesus macaque monkeys can be taught to play video games, which they seem to enjoy doing more for the fun of it than for rewards of food. (They are also apparently competitive, doing better when seated next to another monkey.) They can also learn to choose the higher of two numbers, apply transitive properties to new pairings and select the higher of two abstract quantities represented by letters.

Deborah Blum shrewdly reminds us of such feats in the first two chapters of her new book on the controversies surrounding primate research. Here is the crux of the issue: these animals can communicate with us, reciprocate some kind of emotional attachment, ask us to tickle them, even beg us not to hurt them. Yet other humans (almost never the same ones who communicate with the primates) perform a variety of damaging experiments on them. The benefits of some, although not all, of these experiments — benefits to primates as well as humans — are beyond doubt. We moderns have a hard time seeing tragic dilemmas for what they are: conflicts between two sets of principles, situations in which there is no clear right answer. Participants in the animal-protection controversy who see no validity to the other side's views have chosen to blind themselves for political reasons, putting aside loyalty to the truth.

Through a series of extended glimpses inside the labs and minds of primatologists and a few others, Blum helps us to understand the zealots on both sides of the current controversy, yet she has also found some people capable of at least doubting their own positions. After defending the use of primates in disease research, the chief veterinarian at a large primate research centre admits: "And, yet, I don't feel so right about what I do that I could turn to an animal rights advocate, and say, you are wrong. In the end I think I'm probably wrong." In the heat of the

The cute style and frequent metaphorical descriptions in *The Monkey Wars* betray its origin as a series of articles for the *Sacramento Bee*. But if it has certain analytical limits of journalism, the book represents high-quality journalism — rich details, smooth writing and general accuracy — for which Blum's articles won her a Pulitzer prize. Blum visited half-a-dozen primate research centres and interviewed at least four dozen people involved in the primate-research controversy, most of them active researchers.

Despite some meandering, *The Monkey*



HEAD SCRATCHER — picture from the cover of *Chimpanzee Cultures* edited by R. W. Wrangham et al.. It arose from a 1991 Chicago symposium entitled "Understanding Chimpanzees: Behavior and Diversity". Harvard University Press, \$39.95, £29.95.

clash, such humility has become rare. More often, those who became scientists to search for the truth end up learning that, for strategic reasons, the truth is secondary.

Active suppression, at least, is more the province of private companies than scientists. Perhaps the most egregious case resulted from a 1983 letter to the editor of the *Journal of Medical Primatology* from the head of the International Primate Protection League, criticizing a private company's proposal to study hepatitis in chimpanzees. The company sued, for \$4 million each, the journal, its editor, his university, the writer of the letter, another publication (*New Scientist*) that had run a piece about the proposal, the *New Scientist* writer and the distributors of both publications. All settled out of court except the journal's editor, who won after seven years and \$2 million in legal fees (\$70,000 from his own pocket). The National Association for Biomedical Research, at the forefront of the counter-attack against the animal-rights movement, even filed two briefs on the side of the company. Blum describes other cases in which any questioning of animal use in research is discouraged or punished.

Wars repeatedly returns to the important political issues involved in primate research, usually expressed by Blum's respondents. Beginning with vignettes meant to impress us with the similarities of apes and monkeys to humans, the author describes research on brain structure, stress, deafferentation, virology, organ transplants. Without taking sides, she gives us insights into the worldviews of researchers, animal protectionists and the veterinarians and managers of primate colonies or research centres, and especially the diversity within each group. Two chapters discuss the threat of infectious diseases transmitted from primates to humans, particularly those caused by viruses such as HIV that can sit quietly in other species but then rip through human populations, aided by the genetic proximity. Blum's argument is often submerged in the details, but her point is always clear. The final chapter, after describing a man responsible for 1,600 apes and monkeys who "faults animal activists for making the monkeys too human... scientists for making them too mechanical", crystallizes the essential dilemma of primate research, and warns against easy answers.

This book will change no minds. But it may encourage us to listen to those with less strident and extreme positions, who know a real dilemma when they see it, who refuse easy choices. Among the human primates, at least, they are the main ones who have been voiceless in the controversy so far. An evolutionary virologist, dismayed by the viral risk of organ transplants from baboons to humans, says of a surgeon that he "may say that he sees no real ethical issues here.... But if you don't see them, then you're the wrong person to be making the decision. Maybe this is not an area to be decided by transplant surgeons at all." □

James M. Jasper is in the Department of Sociology, New York University, 269 Mercer Street, New York, New York 10003, USA.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Coming plagues?

Robert Desowitz

The Hot Zone. By Richard Preston. *Random House/Doubleday*: 1994. Pp. 336, \$23, £14.99.

POGO's seminal observation (for those of you who remember Pogo) that "we have met the enemy and they is us" could equally apply to viral biology as to human self-destructiveness. By its insinuation into the host's genome, the virus becomes, in the genetic sense, as much as the host as the host itself. Sometimes this splice of life is clinically quiescent; sometimes it causes an annoying but not life-threatening illness; sometimes it is fatal. And there are two viruses that are man's worst nightmare, Marburg and Ebola.

Marburg and Ebola, filoviruses related to the influenza virus, strike humans with an unparalleled swiftness and destructiveness. The fatality rate is high, especially for Ebola, which kills more than 90 per cent of infected people. The course of the disease is horrible, with the explosive invasion virtually lysing its victim. There is no known cure, and no vaccine or other prophylactic. The viruses are presumed to be zoonotic agents but the natural host, or hosts, remains unidentified. The mechanism(s) of transmission is imperfectly understood. These are viruses surrounded by mystery, the mystery being even more ominous because of the uneasy suspicion that they may have the potential to break out and become "Doomsday bugs".

Marburg and Ebola are, in the literary sense, highly 'photogenic' pathogens that have been awaiting their biographer. Richard Preston attempts to fulfil this role in *The Hot Zone*. He adopts the docudrama format to tell two stories: the people story and the monkey story. The people story begins with a French expatriate

The culprit — Ebola, one of the group of filoviruses and the cause of Ebola fever.

employee of a Kenyan sugar plantation who takes his African girlfriend on a weekend's holiday to Mount Elgon, where they visit Kitum cave. A week later he sickens, becomes progressively more ill and dies, haemorrhaging from all his bodily orifices. A doctor who attends him develops the same symptoms and dies. Blood is drawn and sent to the Centers for Disease Control in Atlanta, Georgia, where a diagnosis of Marburg virus is made. Seven years later, in 1987, a ten-year-old Danish boy visits his parents who work for a Kenyan relief organization. The family goes on an outing to Kitum cave and shortly afterwards the boy falls sick and dies. The US Army Medical Research Institute of Infectious Diseases, Maryland, takes a blood sample and discovers that it is positive for Marburg. The boy's parents and sister are unaffected, as was the Frenchman's girlfriend. An intensive study of Kitum cave and its flora and fauna fails to reveal the virus. Meanwhile, Africans in remote villages in Zaïre are dying of Ebola. Another intensive expedition is mounted but again fails to find Ebola's reservoir.

The monkey story, which makes up the longest segment of the book, describes a killing outbreak of infectious disease in monkeys held in a commercial facility in Reston, Virginia, about ten miles from Washington DC. These were crab-eating macaques imported from the Philippines. US Army virologists diagnose Ebola. But what is this African virus doing in Philippine monkeys and why isn't it killing the animal attendants who develop antibodies to the virus but remain asymptomatic? The US Army comes in and "nukes" the monkey house.

Preston's writing is rich in such 'army speak': the virus containment facility is the "hot zone"; the human containment facility is "the Slammer". The book is also rich in 'pseudo-science speak': the virus

"jumps" and "expands to burn"; the replicating viruses are "blobs" and "fat bricks" bursting from their host cells; the "healthy monkeys went nuts". It is a style of writing that complements the drama of his story. It is also a style of writing that may make you cringe.

The real problem with this book is not Preston's writing style or his lack of adequate insights into viral biology. It is his superficiality and failure to address the immensely important issues that his story illuminates. Does he not get an uneasy feeling, as I do, that military organizations are the custodians of what is considered to be the most lethal pathogen of humans — a pathogen that with a bit of genetic tinkering could become the Andromeda strain? Is he not concerned that there were no contingency plans or laws to enable authorities to deal rapidly and effectively with a potential outbreak of a highly pathogenic agent? Or to prevent the agent from entering the United States in the first place? After the Reston monkey-house disaster the same company imported the same species of monkey from the same place with the same result — virtually all the monkeys died of an Ebola or Ebola-like viral infection. Why does he not ask why there is apparently so little research on so lethal an organism about which so little is known?

For all its shortcomings, this is a powerful book. It will scare the hell out of people. But to my mind the unattended issues of public health and research are almost as scary as the virus itself. □

Robert Desowitz is in the Department of Protozoology, Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. From 15 January 1995 he will be in the Department of Tropical Medicine and Medical Microbiology, University of Hawaii, 3675 Kilauea Avenue, Honolulu, Hawaii 96816, USA.

Darwin and disease

Robin A. Weiss

Evolution of Infectious Disease. By Paul W. Ewald. Oxford University Press: 1994. Pp. 298. \$35, £27.50.

The Evolutionary Biology of Viruses. Edited by Stephen S. Morse. Raven: 1994. Pp. 353. \$87.

I HAVE not picked up a book on infectious disease with so much anticipation as Paul Ewald's *Evolution of Infectious Disease* since reading William McNeil's *Plagues and Peoples* more than 15 years ago. I was not disappointed: Ewald's book is as teeming with ideas as some of us are with microbes. My problem, however, was to discern those ideas that are destined for transmission and further evolution from those that resemble defective interfering notions. Ewald's mission is to persuade us to view infectious disease in evolutionary terms, an approach he calls 'Darwinian medicine'. His main thesis is that the virulence of infectious microorganisms will vary according to natural selection acting on factors favouring their transmission. He reminds us that an agent of infectious disease can quickly replicate in a new host to give upwards of 10^{10} infectious particles, with opportunities for rapid evolution and change in virulence, for example by becoming resistant to antibiotics and drugs. Whereas evolutionists have accepted for a decade or more that infectious organisms can increase or decrease their virulence in the host according to selective pressures, the rest of us still tend to mouth the old saw that the longer the parasite and host live together, the further their relationship will evolve towards benign commensality. Ewald shatters this misconception. He illustrates how "cultural vectors" can predispose to increasing virulence, whether it be diarrhoea from cholera or AIDS from HIV. He goes on to argue that by deliberately manipulating the dynamics of transmission we could reduce the severity of disease, and he makes a plea for more Darwinian thinking in medicine, epidemiology and public health.

Models

Evolution of Infectious Disease is a challenging and readable introduction to current thinking on the topic. As an experimental, laboratory-based virologist, I certainly found it stimulating. Ewald has plenty of pithy aphorisms: he says, for example, that from a pathogen's viewpoint "a dead host and an immune host are not much different". His book is easier going than the excellent modern classic by Robert M. May and Roy M. Anderson, *Infectious Diseases of Humans: Dynamics and Control* (Oxford University Press,

1991). But specialists in the field may find it irritating, for it lacks a certain rigour that one might expect in evolutionary theory and is devoid of any mathematical treatment to explore the robustness of the author's models of disease. Further, the closer Ewald approaches my own field of expertise, the riskier I find his premises and the more suspect his conclusions. This is largely on account of his ignorance of pathology on the one hand, and of molecular biology on the other. I do not think that chronic, persistent infections with, say, the hepatitis-C virus or the human immunodeficiency virus (HIV) can be treated in quite the same way as acute diseases, for which the virulence to the host is more likely to relate directly to their early transmission. For instance, I am not convinced, as Ewald is, that the virulence of human T-cell leukaemia virus type I is bound to increase on account of blood transmission among intravenous drug abusers. And, if it does, would one expect tropical spastic paraparesis to outpace adult T-cell leukaemia because it is more commonly related to parenteral (blood) acquisition?

Mechanisms

Relating virulence to an understanding of pathogenesis would seem to be particularly important in AIDS, yet I was left frustrated by Ewald's chapters on this disease. His account of Kaposi's sarcoma is garbled; he fails to mention the high relative incidence of this sarcoma in other immunosuppressive conditions as seen, for example, in recipients of organ transplants. As to HIV itself, I would have liked to read an evolutionary discussion about the pathogenesis of this newly emerged virus. There are three major views of HIV pathogenesis: that it is an immunologically driven disease, akin to autoimmunity; that virulent subtypes emerge in infected individuals; and that the overall diversity of HIV *in vivo*, rather than particular virulent subtypes, eventually overcomes the repertoire of T-cell responses. Overall, I am not persuaded that virulence to the host relates solely to the dynamics of transmission of the parasite; some knowledge of the mechanism of pathogenesis is needed.

To be fair, Ewald does outline early in his book that some pathological effects of infection may be occasional, dead-end outcomes so far as the invading parasite is concerned. What he does not admit is that many of these 'side-effects', which do not influence the probability of transmission, are nonetheless major targets for improving human health. Take poliomyelitis as an example. The race in the 1950s to develop a safe, efficacious polio vaccine and the current campaign of the World Health Organisation to eradicate polio virus (successfully achieved for the Americas) is driven by such a side-effect, namely

the invasion of the central nervous system, in a minority of infected children. The virus is naturally transmitted by way of the gut and the immobilization of the host through the destruction of motor neurons would not appear to aid transmission. Notwithstanding Ewald's sensible plea for Darwinian medicine, I hold that a significant proportion of mankind's morbidity and mortality, whether through poliomyelitis, cerebral malaria, elephantiasis or 20 per cent of cancers, results from pathologies that are not directly related to the transmission of the causative agent.

Molecular details

The molecular biology lacking in *Evolution of Infectious Disease* is all to be found in *The Evolutionary Biology of Viruses*. Stephen Morse has assembled a series of articles on evolution and on viral variation that represent much more than the usual set of reviews or conference proceedings. Most of the authors have written genuine essays on their subjects, probing the limits of current knowledge and ideas. Every chapter is informative. I benefited in particular from Morse's own introductory article, G. P. Garnett and R. Antia on the population biology of virus-host interactions, E. Domingo and J. J. Holland on mutation rates and rapid evolution of RNA viruses, and E. D. Kilbourne on host determination of viral evolution.

Morse points out that most discussions of viral evolution are generally outside the neo-Darwinian synthesis, and the relationship between viral variation and natural selection remains largely unexplored. In this volume, the processes generating genetic diversity (especially in RNA viruses lacking proof-reading during replication) is brought together with natural selection as a driving force in evolution. This synthesis is timely. A reviewer of a recent paper from my laboratory illustrating how immune escape of HIV can alter tropism (growth in macrophages) and vice versa, stated that there is no reason to think that the immune system exerts any selection on HIV *in vivo*, on the assumption that the generation of variants keeps the virus ahead of the immune system. Such referees should read this volume, in which that veteran evolutionist, Ernst Mayr, reminds us that evolution is a two-step process. The first step produces genetic variation, the second is the process of selection based on altered phenotype. The high mutation rates of many viruses has been noted, writes Mayr, but the individual progeny will later be subject to selection. Quite so. □

Robin A. Weiss is in the Chester Beatty Laboratories, Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK.

Theories in collision

Anthony Hallam

The Mass-Extinction Debates: How Science Works in a Crisis. Edited by William Glen. *Stanford University Press: 1994.* Pp. 370. \$49.50, £35 (hbk); \$17.95, £12.95 (pbk). Distributed outside North America by Cambridge University Press.

In recent years Bill Glen has established himself as a leading figure in what may be described as history of science on the hoof; his crucial piece of equipment is his tape recorder. Following the success of *The Road to Jaramillo*, his esteemed study of the plate-tectonics revolution, he has turned his attention to the mass-extinction controversies, which stemmed from the publication of a seminal paper by Luis Alvarez and colleagues in *Science* nearly a decade and a half ago. A limitation of Glen's earlier study was that he interviewed the principal Earth scientists many years after their important research, so he had to cope with their selective memories and retrospective rationalizations.

By involving himself from the beginning of the mass-extinction debates, Glen has this time avoided such difficulties. He intends to write a book on the subject and the first two chapters of this volume are evidently a precis. Judging by their quality, his full-scale book is likely to be as well received as his earlier work. The first chapter is an excellent review of the rival extraterrestrial-impact and terrestrial-volcanic theories argued for the mass extinction at the end of the Cretaceous period (65 million years ago), and of various outcomes such as the claim for an extinction periodicity, which aroused the interest of several astronomers. The second chapter on "how science works" deals with personal and institutional factors, such as how attitudes to impact and catastrophic events were influenced by scientific discipline, with, for instance, scientists with a strong physical or chemical background being far more sympathetic than palaeontologists, who had greater knowledge of the biological data.

Media involvement

The rest of the book is the outcome of a meeting held in Illinois more three years ago, with a number of contributions varying considerably in quality: in other words, the usual curate's egg. Some are slight, others substantial, some lucid, others, such as Herbert Shaw's, opaque and jargon-ridden; but all in all they give a representative sample of the range of attitudes expressed during the debate. One of the most interesting is by Elizabeth Clemens, a sociologist who makes a plausible case that media involvement relating to the

public's fascination with dinosaurs has driven the debate to a considerable extent. Several Earth scientists who favour impact theories, namely Ken Hsü, Digby McLaren and David Raup, consider that the general resistance of their colleagues to the Alvarez hypothesis, at least initially, was due to their having been indoctrinated with an outdated Lyellian uninformatarianism, which denied the possibility of catastrophic happenings. To which one is tempted to reply, recalling *Scoop*, Evelyn Waugh's delightful novel about journalism, "Up to a point, Lord Copper." If one defines a catastrophic mass extinction as one in which a significant proportion of the Earth's biota becomes extinct in a geologically insignificant period of time, say less than a few thousand years, then it is perfectly possible to believe in such events without necessarily invoking asteroids or comets.

It all depends on the evidence, and only for the mass extinction at the end of the Cretaceous has a strong case been made for impact at that time, in terms of significant iridium anomalies on a global scale, shocked minerals and other data. For all other mass-extinction events, major or minor, the evidence is either ambiguous, inconclusive or nonexistent. In these circumstances, to accept in the absence of supporting evidence impact events as a general cause of mass extinctions, a view evidently favoured by Victor Clube and Shaw, seems to fall in the realm of faith rather than science. Hsü and Shaw invoke fractal geometry, nonlinear dynamics and chaos theory. While it is perfectly true that, as Hsü states, the pattern of natural temporal change shows that the frequency of occurrence of natural processes is inversely related to their magnitude, a phenomenon readily apparent with both floods and earthquakes, and well expressed in Raup's notorious "kill curve", this says nothing about the causal factors.

The 'catastrophists' are very well represented in this book. At the other extreme, John Briggs expresses a more traditional view of extinctions as extended over considerable periods of time, and very rarely of global extent. John Van Valen contributes a thoughtful essay on the relation of extinction to selection and unsurprisingly, as the author of the celebrated Red Queen hypothesis, rejects the view increasingly held by palaeontologists that displacive competition by later-evolved "biologically superior" organisms, as favoured by Darwin, is of far less significance in evolutionary history than preemptive competition by the incumbents of given ecological niches. Only when extinction wipes out these incumbents can other organisms opportunistically respond. So no one seriously argues any more that the dinosaurs died out because of competition from the mam-

mals, with whom they had coexisted through most of the Mesozoic.

One of the most illuminating parts of the book is a couple of interviews by Glen of prominent palaeontologists identified with opposing camps, and who thus present contrasting views. Bill Clemens expresses a balanced, reasonable and well informed scepticism towards the impact hypothesis as an explanation for the extinction of dinosaurs, whereas Steve Gould was converted to the hypothesis fairly early on. Gould is determined, however, to dissociate belief in impact as a cause of mass extinction from his views on punctuated equilibria, a link that Glen persistently tries to establish.

New evidence

So where do we stand today? In the past few years, belief in a bolide impact at the end of the Cretaceous has been strengthened by new discoveries, most notably those related to an apparently huge buried impact crater in the Yucatan Peninsula of Mexico. Although the new evidence is impressive, scepticism is bound to persist in some informed circles at least until one or more new boreholes are drilled to establish the precise age of the oldest sedimentary strata overlying the purported impact debris. This is necessary in order to refute a counter claim by a US oil geologist who worked on the site in the 1960s that several hundred metres of Upper Cretaceous limestone overlies what he and others have interpreted as volcanic rock. But even if one accepts that an impact is conclusively established for the end of the Cretaceous, argument will surely persist about the causal factors of the mass extinction. The earlier 'Dante's Inferno' ideas involving drastic falls or rises of temperature, or both, and acid rain and wild fires on a massive scale, seems far too drastic to account for the selective nature of the extinctions, at least on the continents. It is surprising that so little attention is paid in the book to such factors as Earth-bound climatic and sea-level changes, for there is no reason why these could not also be catastrophic in character on rare occasions. Certainly as a model for mass extinctions in general, they carry greater plausibility than impact events, because they have abundant supporting evidence. As regards sea level, the spread of anoxic bottom waters associated with rapid marine transgressions seems to be a more important factor than the more widely cited regressions. This is indeed likely to be the case for the biggest mass extinction event of all, at the end of the Permian, which, contrary to a widely held belief, evidently had a considerable catastrophic component. □

Anthony Hallam is in the School of Earth Sciences, University of Birmingham, Birmingham, B15 2TT, UK.

Science for the citizen

Graham Farmelo

A new series of books intended to make science accessible to a mass audience has just been launched — but will they repeat the success of Stephen Hawking's *A Brief History of Time*?

THE most depressing thing about today's popular science books is that they are so unpopular. Apart from a handful of blockbusters, very few of the science books written for the general public sell in substantial numbers: according to a recent survey, only two per cent of the UK population have bought a nonfiction book on science or technology within the past 12 months.

The Rhône-Poulenc Prizes for science books have done much to focus attention on fine-quality science writing, notably Steve Jones's *The Language of the Genes*, Jared Diamond's *The Rise and Fall of the Third Chimpanzee* and Stephen Jay Gould's *Wonderful Life*. Yet none of the winners in the prize's eight-year history has sold in vast numbers or established itself in the popular mind as an acknowledged 'good read'. Perhaps the works that are generally praised as gems of popularization are actually examples of excellent writing for other scientists or, at least, for keen and well-informed lay people.

These three attractively packaged books launch the new Science Masters series, which plainly intends to make modern science accessible to a mass audience. This much-heralded series, which is being published in 26 countries, is the ambitious brainchild of Anthony Cheetham, chairman of the Orion Publishing Group in London, and John Brockman, the New York literary agent famous for the lavish advances he is said to have secured for several eminent scientists.

Each of the volumes is short, completely (or almost completely) devoid of equations and written for a wide audience by a leading scientist. The series aims to cater for the "educated but non-specialist reader", which we are told implies that "no prior knowledge of science or mathematics is required". This is an odd statement: can someone be regarded as educated if they know no science at all?

Jargon

Scientific greenhorns will certainly struggle with the accounts of astrophysics given here by John Barrow and Paul Davies, both theoretical physicists. Given their uncommon talent for penning lively and well-informed expositions of the arcana of modern physics, it is surprising how freely they use jargon that will certainly be Greek to those with no training in science (examples: asymptote, ionized plasma, joules and kelvin). Pedagogic justice

would imply that readers will not put up with this, yet sales figures of astrophysics books make it clear that there are many who are prepared to struggle with the most challenging material that the experts throw at them.

This was first demonstrated 17 years ago when the Nobel prizewinning physicist Steven Weinberg published *The First Three Minutes*, his best-selling account of the origins of the Universe. Paul Davies pays handsome tribute to Weinberg's pioneering book and cleverly chooses to focus on the other extreme of the Universe's life, a subject that at first seems to have similar promise. But whereas Weinberg could give a straightforward and dramatic frame-by-frame account of the birth of space, time, matter and energy, Davies has to consider several scenarios of oblivion, including a collision with a comet that ends life on Earth, the big crunch and the so-called 'deep freeze' in which the Universe slides towards degeneration "inexorably" (a favourite word in the Davies lexicon).

Davies takes what is described on the dustjacket as a "free-ranging" approach, delving into dozens of areas in modern astrophysics, several of which nonspecialists may perceive to be peripheral to the main subject. As a result, *The Last Three Minutes* comes over as a concatenation of well-crafted tableaux that collectively have a frustratingly hazy focus.

Davies's account would have been a good deal clearer if he had included the familiar plot of 'size' against time for the various types of expanding Universe. Such a diagram obligingly turns up in Barrow's first chapter, which expertly summarizes "the universe in a nutshell", including the gist of Davies's story. The subsequent parts briskly cover most of the key topics in contemporary cosmology including cosmic ripples, wormholes, black holes and inflation. For those who can stomach the challenge—and this cohort will include everyone who finished Stephen Hawking's *A Brief History of Time* — Barrow has written what is probably the best available short introduction to modern cosmology.

High-quality illustrations are crucial to the appeal of popular science books, so it is disappointing that both Davies and Barrow have been badly let down in this respect. *The Last Three Minutes* features far too few diagrams and the ones that are included are miserably inadequate, to say the least. Yet even these are superior to

most of Barrow's illustrations, which for the most part look as though they have been scratched out using a high-school Indian ink set.

The other member of the Science Masters opening trio, Richard Leakey's agreeable *The Origin of Mankind*, is in every sense more down to earth than its two companions. This clear, plain-spoken account of human evolution covers not only the relationships between ourselves and our African ancestors but also the latest thinking on the development of language, consciousness and economic cooperation. Once again the standard of the illustrations is indifferent—even the chapter on art has only one unprepossessing figure.

Personal view

Leakey's account is pleasingly personal and he occasionally points out where he disagrees with orthodox views, most strikingly when he explains why he dissents from the popular interpretation of Donald Johanson and Tim White of the rich collection of fossils found two decades ago in Hadar, Ethiopia. The reasoning that underlies this and other disagreements will give nonspecialists useful insights into how and why scientists so often view a piece of evidence in different ways. A little more emotion would have added a further touch of veracity.

So the Science Masters series begins well, if not quite as strongly as we might have hoped. Our appetite has, however, been whetted for books in this format and we look can forward to contributions from Richard Dawkins, Daniel C. Dennett, Colin Blakemore and a host of other luminaries. One hopes that it will not be long before the publishers recruit Freeman Dyson, one of the most graceful essayists to have written on physical science. Meanwhile, will someone talk to whoever is responsible for the series' graphics? □

Graham Farmelo is Head of Programmes at the Science Museum, Exhibition Road, London SW7 2DD, UK.

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The Last Three Minutes. By Paul Davies. BasicBooks/Weidenfeld and Nicholson: 1994. Pp. 162. \$20, £9.99.

The Origin of the Universe. By John D. Barrow. BasicBooks/Weidenfeld and Nicholson: 1994. Pp. 150. \$20, £9.99.

The Origin of Humankind. By Richard Leakey. BasicBooks/Weidenfeld and Nicholson: 1994. Pp. 171. \$20, £9.99.

A case of curiosities

Richard Davenport-Hines

Henry Wellcome. By Robert Rhodes James. *Hodder and Stoughton: 1994.* Pp. 422. £25.

THE old-fashioned phrase 'a man of parts' might have been coined to describe Henry Wellcome (1853–1936), pharmaceutical multimillionaire, collector and philanthropist. The son of a farmer and fanatical itinerant preacher, he was born in a frontier settlement in Wisconsin and educated in a log-cabin school in Minnesota and at the Chicago School of Pharmacy. From an early age (perhaps as a reaction to the dyslexia and religious bigotry that ran in his family) he evinced "hero-worship of better minds than his own, and . . . fascination with ideas and intellect". As a salesman he travelled all over the Americas in the 1870s, peddling medicines with furious energy as a way to escape from boyhood poverty. In 1880 he became the junior partner of an Englishman, Silas Burroughs, specializing in a line of products with the tradename Tabloids. Their relationship later became very strained, but attempts to dislodge Wellcome from the business failed, and he took control of the company on the death of Burroughs in 1895.

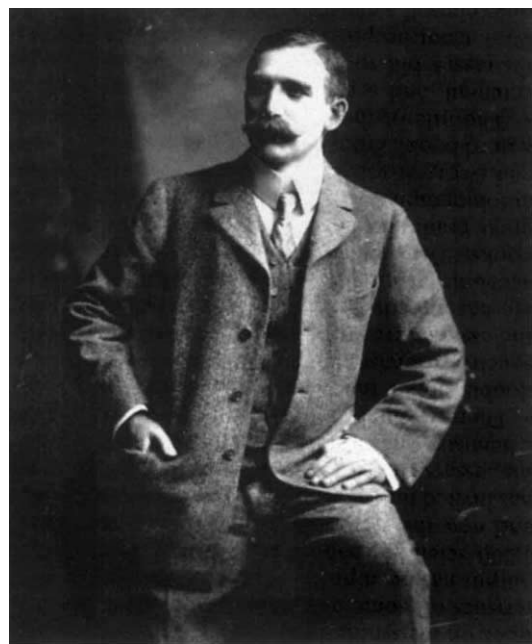
Over the next two decades, Wellcome proved himself to be a keen, resourceful and flexible businessman who pushed the Burroughs Wellcome company to the forefront of the British pharmaceutical sector. His greatest contribution to British pharmaceutical history was to set up the semi-independent Wellcome Physiological

Research Laboratories with the highest standards of research and quality control. The researches of pharmacists had hitherto been tainted as dishonest quackery, but it was Wellcome's achievement to remove suspicion of 'commercialization' from scientific work. He also expanded the overseas operations of the company, which after 1914 made a crucial contribution to sera and vaccines needed during the war.

Comparison with the other great pioneering leader of British pharmaceutical enterprise, Sir Harry Jephcott of Glaxo, is instructive. Jephcott was originally a research scientist who worked his way up in a company owned by others. He had all the qualities of a company insider. He was discreet, modest, unneurotic, leading his employees by example rather than intimidation, and was understandably adored by many of them. Wellcome the founding entrepreneur was neurotic, turbulent, flamboyant, self-centred and often destructive in his effects, although his instincts were generous; he had few friends, and was respected rather than admired. Both men had exceptional tenacity, energy and a powerful instinct for research strategies and their commercial possibilities.

As well as being an innovative entrepreneur, Wellcome had a mania for collecting curios, a passion that contributed to the failure of his marriage to Syrie Barnardo, daughter of the medical philanthropist who founded the Barnardo Homes for orphans. He amassed a huge collection of objects intended for the Wellcome Historical Medical Museum which he founded in 1913.

His collection was very idiosyncratic: after his death it was found to include items as disparate as a rose from the garden at Khartoum of General Gordon, the wheelbarrow, cord and trapeze with which Blondin crossed the Niagara Falls, more than 80,000 models of patents registered in the US Patent Office that had never been uncased, 17,000 amulets, 52 cases of flints and 4,000 spears. His historical collections were not intended for the general public ("stragglers", as he called them); rather, they were intended to serve as a laboratory where cultural and technological problems would be solved, an activity



Henry Wellcome in 1902.

to which he devoted increasing amounts of time to the detriment of his day-to-day business operations.

In 1924 Wellcome consolidated his separate enterprises — nine companies, his research laboratories and museum — in one private company, the Wellcome Foundation, control of which he bequeathed to trustees. Under the terms of his will he subdivided his empire into the Wellcome Foundation, operating its pharmaceutical business, and the Wellcome Research Institution, funded by the former's profits and pursuing a range of chemical, physiological, entomological, archaeological and historical research. Thus, confusingly, the Wellcome Trust (established by his will) became the real 'foundation' charged with distributing the income from Wellcome shares — the Wellcome Foundation acts as the commercial arm. He also tried to impose complex and sometimes inoperable instructions on his managers from beyond the grave. His instructions to his trustees were absurdly grandiose and led to decades of legal wrangling.

Sir Robert Rhodes James, a retired member of the British parliament, has written an elegant, readable and meticulously researched biography of this sad tycoon. Rhodes James is never sycophantic and does not flinch from showing his subject's arrogance or dismal old age, but the overall mood of his book is generous and broad-minded. He touches on issues such as the commercialization of science and charitable administration, but above all gives a vivid study of practical idealism and of the megalomania that develops from the isolation of great riches. □

Richard Davenport-Hines is at 51 Elsham Road, London W14 8HD, UK.



A medical and sanitary exhibition, London, 1881.

Bitter pills

R. V. Short

From the Lab into the World: A Pill for People, Pets, and Bugs. By Carl Djerassi. American Chemical Society: 1994. Pp. 230. \$24.95.

SAMUEL Pepys described himself as having "a fine conceit", and no doubt Carl Djerassi would use the same epithet for himself. But perhaps we can forgive him this high opinion, because he has certainly achieved more than many of us could do in several lifetimes. As mentor to an annual army of 20 or more postgraduate and postdoctoral students in the department of chemistry at Stanford University, and sometime president of Syntex Research, he has always had his feet planted firmly in two camps, and has been driven by the need to find practical applications for laboratory discoveries. Working in a rival laboratory in New York in 1962, I can still remember our incredulity at Djerassi's scientific output, with almost a publication a week on the synthesis and spectral characteristics of some new steroid. The man's output has been prodigious — more than 1,200 scientific papers and 13 books — and now the American Chemical Society has invited him to republish a collection of his essays in its *Creators of Modern Chemistry* series.

Djerassi himself obviously had some doubts about the wisdom of this retrospective exercise, particularly as some of the essays were written in the early 1970s. He has already given us detailed popular accounts of his life's work in two of his books, *The Politics of Contraception*, a great read, and his less enjoyable recent autobiography *The Pill, Pygmy Chimps, and Degas' Horse*. So was there a need for a third book? Probably not.

The title of the new work is a bit of a catch-all, and seems designed to further Djerassi's bid to be remembered as Mr Oral Contraceptive Pill. But herein he does himself a disservice; the judgement is best left to others, and the accolade unquestionably goes to Gregory Pincus of the Worcester Foundation in Shrewsbury, Massachusetts. Djerassi admits that when he and his colleagues at the Syntex laboratories in Mexico City synthesized an orally active progestagenic steroid, norethindrone, in October 1951, the idea that it might be used as one of the components of an oral contraceptive pill had not even crossed their minds. Yet it should have done, for the concept of using progesterone to inhibit ovulation was well known to biologists in the 1930s and 1940s. It was Pincus, a biologist, who realized that it was necessary to combine an orally active oestrogen with an orally active gestagen in order to inhibit ovulation and control

menstruation, and it was he who organized the clinical trials that led to the eventual marketing of the first combined oral contraceptive pill. But Djerassi is understandably aggrieved that Pincus failed to acknowledge his indebtedness to the chemists such as Djerassi and others who provided him with a range of orally active oestrogens and gestagens. If one message comes out of this book, it is that progress could be accelerated if chemists had more biological insight and biologists were more aware of what chemists had to offer.

Djerassi will be best remembered for his landmark publication in *Science* in 1970 entitled "Birth Control After 1984", and it is good to see it reproduced here. Written from the viewpoint of the pharmaceutical industry, it described critical pathways for the development of a hypothetical abortifacient for women and an antifertility agent for men, and concluded that neither would be widely available in the next two decades. Although we knew all about steroidal antiandrogens and antioestrogens well before 1970, there had been no concerted effort by chemists to develop an antigestagen, and RU486 (mifepristone) was discovered by Roussel-Uclaf scientists in Paris only by accident while they were searching for an anti-

glucocorticoid. It is being used increasingly in Europe and China in conjunction with an oral prostaglandin for aborting early pregnancies, and women seem to prefer a medical as opposed to a surgical termination. But if biologists and chemists had been in closer communication with one another, surely the development of this much-needed abortion pill could have been greatly accelerated.

Djerassi's book concludes with a delightful, unexpected and moving chapter on art patronage. Tragically, his daughter committed suicide; "it took my daughter's suicide", Djerassi says, "to make me take seriously the patronage of the living". So he established a residential centre in California for visiting artists. Set in 600 acres of wooded country in the Santa Cruz mountains, overlooking the Pacific Ocean, it has become a place of inspiration for writers, painters, sculptors, photographers, composers and choreographers since 1979. For that, he will be long remembered, when the 1,200 scientific publications that made his philanthropy possible are long forgotten. □

R. V. Short is in the Department of Physiology, Monash University, Melbourne, Victoria 3168, Australia.

The bell curve revisited

Adam Kuper

The Evolution of Racism: Human Differences and the Use and Abuse of Science. By Pat Shipman. Simon and Schuster: 1994. Pp. 318. \$23.

THIS book, which tells the story of biological research on race, is certainly timely. The US media are going through one of their periodical convulsions about race and IQ, sparked off by the publication of *The Bell Curve* by Charles Murray and Richard J. Herrnstein. *Newsweek* made the controversy a cover-story, *The New Republic* devoted an entire issue to it and *The New York Times* ran turgid attacks on the authors virtually every day for a month. President Clinton said he had not read the book, but that he disagreed with its conclusions.

The reason for all the publicity is, of course, that, notwithstanding its parade of graphs and tables, *The Bell Curve* packs a controversial message about education policy (one cannot compensate for having the wrong genes) and, inevitably in the United States, this implicates race. The fuss will have come as no surprise to Pat Shipman. She shows that research on race always stirs up political passions. The scientific arguments are also wearisomely familiar. Discussing earlier controversies, she patiently and clearly reviews the

established and sound arguments that critics are now bringing in turn against *The Bell Curve*. It is notoriously tricky to define intelligence, and very hard indeed to design a measure of intellectual skills that is not biased in favour of children raised in middle-class, English-speaking, non-immigrant families. The racial categories (Caucasoid? White? Nordic? Mediterranean? Jewish?) are hopelessly crude. And environmental effects are, as always, treated as a stable residual factor, despite the fact that, for example, the famous US Army IQ tests during the Second World War revealed that blacks from states with a decent educational system scored better than whites from states with a poor educational base. The argument about race and intelligence has been long, bitter and fruitless, and it is certainly worth recalling all the other bitter and largely inconclusive arguments that echo through current controversies.

Shipman begins her story with the Darwinian revolution (rather than, for instance, with the father of racial science in Britain, J. C. Pritchard), evidently because this is, for her, the moment when true science enters the lists; and she gives a vivid account of the emergence of evolutionary theory. But she might have said more about Darwin's own, changing view of the human races, and his notions about

the role of sexual selection rather than natural selection in their genesis. The public controversy she chooses to highlight here is the one between T. H. Huxley and the Christians, but that was not about race, and she might have done better to consider the debates, in which the Darwinians were embroiled, between the rival Anthropological and Ethnological Societies, which were divided not only on the scientific issues but also by their views on slavery. This would have brought out the fact that Darwin and Huxley, at any rate, were never naive about the political implications of their theories.

The story then shifts to Germany, where the protagonists were Ernst Haeckel, a Darwinian but also a romantic nationalist and an early scientific exponent of the Aryan race myth, and Rudolf Virchow (whose trenchant arguments against a Darwinism then still innocent of genetics are rather skimmed). Haeckel became a champion of eugenics, revered in due course by the Nazis, and some of the leading German scientists travelled the terrible road from eugenics to an endorsement of the Final Solution.

In the 1930s and 1940s, the political programme of human domestication was forever discredited by the Nazis. Its scientific basis had also been undermined by the new evolutionary synthesis of Mendelianism and Darwinism. Shipman captures the post-war mood in her account of Julian Huxley's virtuoso campaign at UNESCO (United Nations Educational, Scientific and Cultural Organization) against racist thinking. Before the war he had ridiculed the stereotype of the Aryan: "as blond as Hitler, as dolichocephalic as Rosenberg, as tall as Goebbels, as slender as Goering, and as manly as Streicher". Julian Huxley had played a distinguished part in the formulation of the evolutionary synthesis, and although once an advocate of eugenics and frankly antisemitic, he now insisted that "races" were social constructs rather than biological entities (phenotypical features being a poor guide to the constitution of the inner man, and migration and interbreeding in any case hopelessly confusing the genetic picture).

There was a similar reaction within US anthropology. Sherwood Washburn, Theodosius Dobzhansky and Ashley Montagu led the charge against the typological thinking in the name of the evolutionary synthesis. The scientific debates were engulfed by political emotions as the civil-rights movement gained momentum, and there were ugly confrontations. Shipman tells with sympathy the story of the humiliation of one of the old guard, the race typologist Carleton Coon. She dismisses the widespread belief that he knew very well what political capital would be made from his speculations, but she is less charitable to his opponents, imputing

political motives for their rejection of race as a significant element in human biology.

Having illustrated the unending interplay of the science and politics of race, Shipman draws an unexpected moral: the frontier between science and politics must be more strictly policed. Scientists should press on with their studies without bothering about the vulgar abuses to which their research might lead. She quotes but dismisses rather too easily the view of an Afro-American intellectual that "there are types of research that shouldn't be done, and the grounds on which I argue are civility". We must, she tells us, continue to do research into human differences.

But there is no way to prevent public debate on any research that impinges on race, or on the biology of difference more generally. As she shows, even research proposals can become national political issues. Moreover, there is still little to suggest that this is a line of research that will yield important new insights. Shipman

implies that there are significant differences between populations that may be explained by a more modern, more sophisticated, more nuanced theory of race, or perhaps by the discovery — at long last — of genetically controlled differences in personality, or sexual disposition, or proclivities to violence or perhaps even rationality. So far, these associations remain highly speculative. (Oddly, she ignores human sociobiology, which has pursued this grail for 20 years, albeit without notable success.) Only one thing seems to have been established beyond any doubt, as her book makes very apparent. From the debates between the Darwinians and their opponents in the 1860s to the clashes over *The Bell Curve*, it has proved impossible to insulate theories about race from their political implications. □

Adam Kuper is in the School of Social Studies, Institute for Advanced Study, Olden Lane, Princeton, New Jersey 08540, USA.

Huxley goes to Hollywood

W. F. Bynum

Huxley: The Devil's Disciple. By Adrian Desmond. *Michael Joseph: 1994. Pp. 475. £20.*

HE would, wouldn't he? Adrian Desmond, fresh from the coauthored blockbuster study of Charles Darwin, has turned to T. H. Huxley as a natural sequel. The remarkable thing is that the companion piece has appeared so soon. Those who liked *Darwin*, which Desmond wrote with James Moore, need have no fear. *Huxley* is in the same mould. It has the same powerful style and the same penchant for florid language and colourful imagery. It is also based on a comparable mass of archival research, unobtrusively

documented. Like *Darwin*, *Huxley* is a jolly good read.

In a brief prolegomenon, Desmond identifies the guiding principle underlying both of these highly imaginative biographies. "*Huxley* uses a 'ciné theory' of narration, with its historiography hidden, to conjure up a flesh-and-blood picture of T. H. Huxley", he tells us, citing as the technique's authority a forthcoming article by Moore. In practice, this means that the reader is invited to forget everything that has previously been written about Huxley and pretend to be there with him at home and abroad, to witness the development of the *Devil's Disciple*. Desmond's narrative rarely looks ahead, rarely pauses to take stock, never explicit-



Huxley rages against the *Pall Mall Gazette*, as J. Tyndall looks on.

From Huxley

ly considers what other scholars have made of this or that episode, confrontation or discovery. Desmond's camera is almost always just behind Huxley's shoulder.

Cynics might suggest that cine theory is suitable only for tabloid biographies and romantic novels (or films): that it is the dominant technique of the recent biographies of Nancy Reagan and the Princess of Wales. Nevertheless, the technique works here for Desmond, for two reasons. One is simply his integrity. He stays close to his sources so that almost every paragraph in the whole volume contains one or more quotations, mostly from Huxley's correspondence. These give his narrative the immediacy that the cine theory requires. Huxley had execrable handwriting but a wonderful way with words. Desmond deserves a prize merely for taking Huxley on.

The extent of the archival remains (more than 5,000 letters in the Huxley Archive at Imperial College, London) is the second reason why Desmond could write the kind of biography he has. It would be impossible to do the same for George Busk, W. B. Carpenter or some of Huxley's other close friends. It might even be difficult for Richard Owen, whose papers were carefully weeded by his grandson. Desmond, the silent cameraman, never offers us his reflections on gaps (deliberate or accidental) in the Huxley Archive.

Despite the fullness of his sources, Desmond occasionally slips into the novelist's mode, even in the very opening paragraph of the book:

The lanky 15-year-old sidled down fetid alleys, past gin palaces and dance halls. Sailors hung out of windows, the gaiety of their boozy whores belying the squalor around them. The boy's predatory looks and patched clothes seemed in keeping. But his black eyes betrayed a horror at the sights: ten crammed into a room, babies diseased from erupting cesspits, the uncoffined dead gnawed by rats. The scenes would scar him for life.

This is powerfully evocative, almost certainly poetically true and maybe even literally correct. We can never be sure. Here, as elsewhere, Desmond the cameraman becomes Desmond the director. He asks, therefore, for a lot of trust. It is amply rewarded. The flesh-and-blood Huxley who emerges from this book is eminently believable, more fascinating in his own right than he could ever be simply as Darwin's Bulldog. Indeed, in many ways, Huxley is a more appropriate character for Desmond's sensibilities than the rich, remote, neurotic Darwin. We are told on the second page (and subsequently reminded several times) that Huxley was born over a butcher's shop. He was a self-made man, surrounded by free-falling relatives, forced to scrimp and borrow his



Black-eyed Tom in 1846.

way through medical school at Charing Cross Hospital, London, unable to afford the complete set of examinations that could have lead to a career as a consulting surgeon. Instead, he chose the even thornier path: to become a professional scientist. Unlike many upwardly mobile individuals (a recent British prime minister springs to mind), Huxley never turned mean, never ceased to be indignant that a rich country could permit so many of its citizens to remain poor.

There are many good things in Desmond's book. Two in particular stand out. The first is his reconstruction of Huxley's years as assistant surgeon on HMS *Rattlesnake*. Almost as long (40,000 miles, and two months short of four years) as Darwin's *Beagle* trek, this voyage was the making of Huxley. It committed him to science, established his reputation and taught him to love, even if his marriage had to wait another four years. No one has ever charted Huxley's experiences so fully, or shown how formative they were.

Desmond's second outstanding achievement is to document the sea-change of public opinion between about 1855 and 1870. The *Origin of Species* provided a turning point, of course, but looking through Huxley's rather than Darwin's eyes provides a broader vision. In those years, professional secular science was established in Britain. Huxley's generation came to maturity. He, John Tyndall, George Busk, Edward Franklin, Joseph Hooker, William Flower, John Lubbock and others of like mind found themselves in positions of power within the British scientific establishment, and with friends in high places in government. Huxley's was not simply a fight *Für Darwin*; it was a

battle on behalf of the social and intellectual value of scientific enquiry. The military metaphors were Huxley's.

Historians have often revelled in the irony that Darwin's Bulldog never really appreciated the explanatory power of natural selection, and took almost a decade even to value Darwin's ideas on genealogical taxonomy. Desmond deftly handles Huxley's relationship to Darwin by showing that, for Huxley, the stakes were higher than the contents of any one book, no matter how great.

In 1869, Huxley was elected president of the British Association for the Advancement of Science. Desmond takes him to his 1870 presidential address and, then, without explanation, leaves him. There is not a single word on the last 25 years of his eventful life, nor any hint of a second volume. The camera simply runs out of film. We are thus deprived of the American tour, the continuing educational activities, the presidency of the Royal Society, the altercation with Prime Minister William Gladstone and the remarkable Romanes lecture. At least 1870 catches Huxley's intimate involvement with the foundation of a new weekly journal. "What a glorious title, *Nature*. It is more than cosmos. More than Universe". The words were not Huxley's, but they could have been. □

W. F. Bynum is in the Wellcome Institute for the History of Medicine, 183 Euston Road, London NW1 2BE, UK.

Sociological searchlights

John Ziman

Handbook of Science and Technology Studies. Edited by Sheila Jasanoff, Gerald E. Markle, James C. Peterson and Trevor Pinch. SAGE: 1994. Pp. 820. \$85, £65.

It was not until the 1960s that people interested in studying science began to realize that it was, above all, a social institution. Turning away from philosophy towards sociology, they opened up a vast new field of research. The annual meetings of the Society for Social Studies of Science ('4S'), which was founded about 20 years ago, are now the marketplace for a bewildering variety of academic wares. In 1988, the society set up a committee to prepare this comprehensive guide to their products. Here, in a pumpkin rather than a nutshell, is what STS — science and technology studies — is supposed to be about.

This fat volume is best read as a collection of 28 well informed local surveys of a

widely dispersed research field. Even when writing in pairs, the 41 contributors are too much themselves to conform to an orderly overall scheme. Chapters that are excellent in their own right take off in various directions, and are not cross-referenced. The earnest editorial effort to relocate them on a general map is no more successful than usual in such works.

The subject as a whole is its own best map. In everyday discourse, science and technology are merged into a single entity with conventionally distinct aspects. One can guess pretty well what will be covered in chapters with titles such as "Women and Scientific Careers", "Science and the Media", "Science as Intellectual Property" and "Science, Technology and the Military". Taken separately, each of the various chapters is a thoughtful and authoritative overview of the sort of thing that is said on such a topic at a 4S meeting. Taken together, they cover reasonably completely those parts of the STS area that happen to be well represented at such meetings.

Unfortunately, this leaves unstudied many of the most important aspects of science and technology. The key word in 4S is "social", yet sociological searchlights are not directed into the relevant institutions. Universities, libraries, learned societies and publishing houses are taken for granted, or castigated, rather than coolly scrutinized.

Sensitive social practices such as peer review are treated as politically suspect rather than, say, functionally expedient.

The social 'interests' that are said to motivate scientific research and technological development lack personal or economic dimensions. Three separate chapters of concern about the obstacles to research careers for women are not embedded in the general social psychology of science as a profession. School science is dismissed perfunctorily, with no mention of the educational interpretation of STS as science, technology and society.

The historical component of STS is often considered to be an independent discipline. In any case, it is too diffuse and bulky to have been summarized in this handbook. That is no excuse for ignoring today's changes in the way in which scientific research is performed. There can be little sense in any study of science or technology that fails to take account of the transformation of laboratory and lecture-room life, right down the line, by new policy agendas, more demanding funding arrangements, increasing teamwork and networking, heavier instrumentation, and other manifestations of the "collectivization" of what was traditionally an individualistic culture. How is it that 'Big Science' does not get any index entries? In the global dimension, surely it is significant that they order such matters very differently in France, Germany, Russia, Japan — or Indonesia, or Costa Rica.

These inadequacies might be acceptable if the disconnected pragmatic map were implicitly unified by a deep principle. But Michel Callon, in his brilliant introductory survey of "Four Models for the

Dynamics of Science", rejects the possibility of formulating such a principle. This postmodern distrust of 'foundationalism' must be right. Science and technology are too protean to be represented by some simplified model purporting to rest solidly on metaphysical bedrock. STS is a field where the fruits of a hundred flowers should be gathered.

The contributors are of one mind only in sharing the notion that everything to do with science and technology is 'socially constructed'. Liberally interpreted, this is a truism. More narrowly, it reminds us to look closely into the interpersonal, organizational and cultural factors that shape the making and use of knowledge. Those factors are extraordinarily rich, amazingly diverse and provocatively puzzling. We lack a general theory covering all human thought and practice. The social elements can enter only piecemeal in our attempts to understand a variety of different contexts of thought or action. Sometimes we can make sense of a situation by using one mode of social interpretation, such as economic metaphor; at other times quite a different mode of social being is involved, such as linguistic participation in a shared life world.

Callon's warning against dogmatism is tacitly ignored. In spite of radical aspirations, highbrow pretensions — and much very stimulating research — the STS community has sharply limited the scope of its constructive imagination. On the fundamentalist wing, the devotees of SSK — the self-styled sociology of scientific knowledge — not only discount the research claims of the scientists; they also spurn insights and critiques from 'non-social' disciplines such as analytical philosophy, evolutionary biology or cognitive psychology. In this way they disengage themselves from some of the most insistent issues of scientific practice and policy. The liberating notion that knowledge is socially constructed has degenerated into an incantation that sweeps the perennial problems of epistemology, ethics and historical change under a pliable carpet of cultural relativism. No wonder that most thoughtful natural and social scientists are neither amused nor enlightened by such intellectual caprices.

Science and technology are much too serious to be left to scientists and technologists. Independent studies of science and technology are an imperative political, industrial, social, cultural and scholarly need. This handbook is designed to attend to this need, but does so only very partially. There is much, much more still to be done. □

John Ziman, emeritus professor of physics at the University of Bristol, is at 27 Little London Green, Oakley, Aylesbury, Buckinghamshire HP18 9QL, UK.

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